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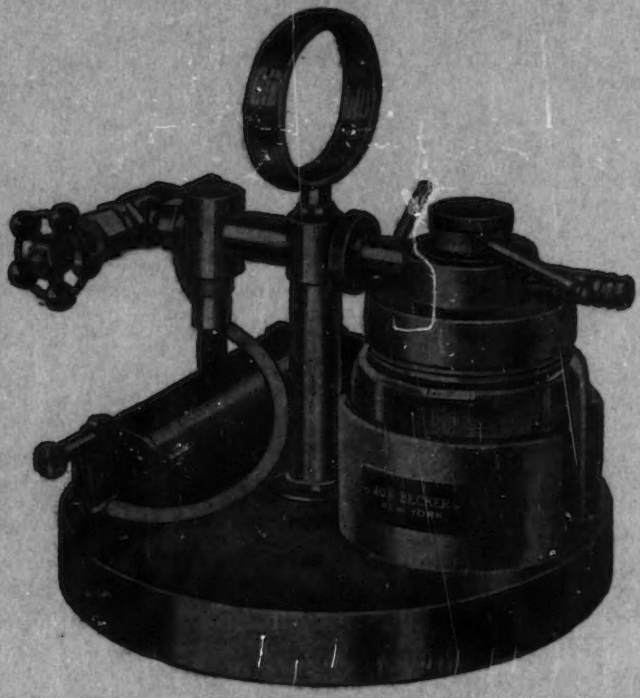

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A STUDY OF CERTAIN FACTORS CONCERNED IN THE SPECIFIC DYNAMIC ACTION OF AMINO ACIDS ADMINISTERED INTRAVENOUSLY AND A COMPARISON WITH ORAL ADMINISTRATION

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In our previous studies on the specific dynamic action of certain amino acids (8), (9), (10), (11), the amino acids have been administered intravenously. The choice of this method of administration rested primarily on the fact that by this means we were able to introduce a known quantity of amino acid into the body in a known period of time. On purely hypothetic grounds the oral method of administration may be considered as the most desirable, since it more closely simulates the natural conditions, but this theoretic advantage is definitely overshadowed by two marked practical disadvantages: 1, the rate and quantity absorbed may vary considerably for different amino acids (3), (12), and presumably for the same amino acid in the same animal under different conditions, thus introducing a factor which may be difficult to evaluate in the intact animal; and 2, during fasting, phlorhizination, following hepatectomy, and so forth, the animals may vomit any solutions given by mouth. These disadvantages are sufficiently serious to limit the number and type of experiments which can be performed under these and other conditions when oral administration is employed, but are to a large extent avoided when the amino acids are given intravenously.

Before we are entirely justified in considering the results which we have previously obtained, employing intravenous administration, as indicative of fundamental principles on which superstructures of generalization could legitimately be erected, it is absolutely essential to be certain that the direct introduction of the amino acids into the peripheral venous blood stream calls forth a response which is physiologically comparable to the

response obtained when the same amino acid follows the natural path and enters the portal blood from the intestine. It is our purpose in this paper, first to present a summary of certain experiments which have been performed during the last three years, in which we have studied various factors which might presumably influence the response of the organism to intravenously administered amino acids, and second, to present comparable experiments on the same animals with both the oral and intravenous methods of administration of the same amino acid.

METHOD OF EXPERIMENTATION. The method of indirect calorimetry employed is that described by Boothby and Sandiford and adapted to use in animals by Kitchen. Briefly, the method consists of the collection of expired air in a gasometer for periods of ten minutes with intervals of three to five minutes between successive collections. Samples of expired air are then analyzed in duplicate with the Haldane gas analysis apparatus for the percentage of carbon dioxide and oxygen. Duplicate analyses must agree within 0.04 per cent for oxygen and 0.03 per cent for carbon dioxide; if these requirements are not fulfilled, a second set of analyses is made. While the tests are being run the animals are kept at a constant environmental temperature between approximately 25° and 30°C., depending on the particular animal used. The animals breathe outside air which is warmed to approximately 25°C.

On the morning of the experiment, the animals were catheterized and the bladder was washed with a constant, measured volume of physiologic saline solution. During the next two hours six satisfactory tests of the basal production of heat were made. At the end of this period, the animals were again catheterized and the bladder was washed, without removing the mask or disconnecting the animal from the gasometer. The solution to be injected was made up to a volume of 50 cc., warmed approximately to body temperature, and injected into the saphenous vein. Collections of expired air were taken during the injection and continued for approximately four hours after the start of the injection. During the first hour after the injection, the interval between successive collections was three minutes, and was five minutes for the remaining three hours. Approximately two hours after the injection, and again at the termination of the experiment, the bladder was emptied and washed. Three samples of urine collected over periods of two hours each were thus obtained, the first representing the basal period, whereas the remaining two covered the period of four hours after administration of the amino acid or other solution. The samples of urine were analyzed for total nitrogen, urea plus ammonia nitrogen, ammonia nitrogen, and amino acid nitrogen, by the methods of Kjeldahl and of Folin.

We have previously shown (9), (10) that the response of the same animal to the intravenous injection of certain amino acids can be greatly altered

by changing the nutritional condition of the animal. The animals employed in these experiments had been receiving a weighed quantity of standard diet[1] at the same time each day, and, with a few exceptions, all were being maintained on approximately the same nutritional level. The animals usually received an amount of the standard diet sufficient to cover the actual basal heat production, plus 50 per cent additional for activity. This quantity of diet usually maintains the animals in constant weight at a low or intermediate nutritional level. The animals are kept in small individual cages at an environmental temperature of approximately 70°F. The importance of constant dietary and environmental conditions can hardly be overestimated and when these are disregarded comparable and constant results, in the same and in different animals, cannot be obtained. Many of the conflicting results to be found in the literature are possibly to be explained on this basis.

METHODS OF CALCULATION. The values for consumption of oxygen and production of carbon dioxide as obtained in successive tests of ten minutes' duration, with intervals of three to five minutes between tests, were expressed as liters per hour and plotted on coördinate paper as ordinates, with the time in hours as abscissas. The average hourly values for the six tests performed during the period of two hours before injection were considered as representing the constant basal consumption of oxygen, and production of carbon dioxide. Zero time on the charts corresponds with the beginning of the injection, and the individual tests have been plotted to the midpoint of the period (that is, the ten-minute test obtained during injection is plotted as five minutes from zero time). The area of the space lying between the basal line for consumption of oxygen or production of carbon dioxide, and the line representing the value obtained after injection of the amino acid or other substance, was then determined with a standardized planimeter and was converted into liters; these values represent the extra consumption of oxygen or production of carbon dioxide. When the basal values per hour are multiplied by the length of the experiment, in hours, after the beginning of the injection, and added to the extra values, as obtained, the results represent the total consumption of oxygen and production of carbon dioxide for the approximate period of four hours after injection.

In calculating the amount of amino acid deaminized and excreted unchanged, as well as the protein metabolism, we proceeded as follows: Following the injection of the amino acid the increase in urea and ammonia nitrogen above the basal level of excretion was considered as representing the amount of amino acid deaminized. Similarly the increase in the amino

[1] The standard diet consists of 44 per cent ground, fat-free beef heart; 44 per cent cracker meal; 8 per cent lard, and 4 per cent bone ash.

acid nitrogen above the basal value was considered as representing the amount of amino acid excreted unchanged. The total nitrogen in grams per hour excreted during the basal period, before injection, is considered as representing the protein metabolism for that period only. The entire quantity of total nitrogen excreted during the four-hour period following injection of the amino acid, minus the extra urea plus ammonia nitrogen and the extra amino acid nitrogen, was considered as representing the protein metabolism after injection of the amino acid. In calculating the nonprotein, nonamino acid respiratory quotients, we employed the usual factors for protein (5) while the factors used for alanine and glycine were those previously employed (8). The nonprotein respiratory quotient, and the total calories for the basal period, were calculated on an average hourly basis; following injection of the amino acid or other substance the nonprotein respiratory quotient and the total calories were calculated for the entire period of four hours. The basal calories per hour, when multiplied by the exact duration, in hours, of the experiment after the injection, show what the production of heat would have been had no injection been given, and the difference between this value and the production of heat calculated for the period of four hours after injection represents the specific dynamic action, or increase in metabolism, brought about by the substance injected.

In the control experiments in which the normal metabolism was determined for approximately six hours, with catheterization every two hours, but with no injections, the same general methods of calculation were employed; the first two hours were calculated on an average hourly basis, and the total production of heat and respiratory quotients for the remaining four hours were calculated as has been described. The production of heat per hour during the first two hours, multiplied by the exact duration of the remainder of the experiments, was considered as the basal value, and the total production of heat for the remaining four hours was then compared with this value. When calculated in this manner, the control experiments are strictly comparable to the experiments in which amino acids or other substances were injected at the end of the basal period of two hours. When physiologic saline solution or solutions of glucose were injected, there frequently occurred an increase in urea plus ammonia nitrogen or total nitrogen, possibly due to a washing-out process; when this occurred the extra urea plus ammonia nitrogen was subtracted from the entire quantity of total nitrogen excreted after the injection and the remainder was considered as representing the true protein metabolism. Whether or not this procedure was justified may be open to question, but the change in the final figures is of negligible magnitude.

METABOLISM OF NORMAL DOGS FOR PERIOD OF APPROXIMATELY SIX HOURS. These results are presented not only as controls for the particular animals used and for the present series of experiments, but in a larger sense to

serve as controls for other work in the laboratory, since similar results have been obtained on all of the perfectly trained animals which we use in accurate quantitative studies. We believe that accurate quantitative results cannot be obtained unless the methods and animals employed are capable of showing a similar constancy of basal metabolism over similar periods of time. In order to avoid any misunderstanding it is important to realize that constant results of the type shown could not be obtained on unselected animals, since many of them cannot be trained to relax completely and breathe regularly; in our work, however, we select only those animals that are capable of being perfectly trained and giving constant results of this nature. Special training of the animal is of equal impor-

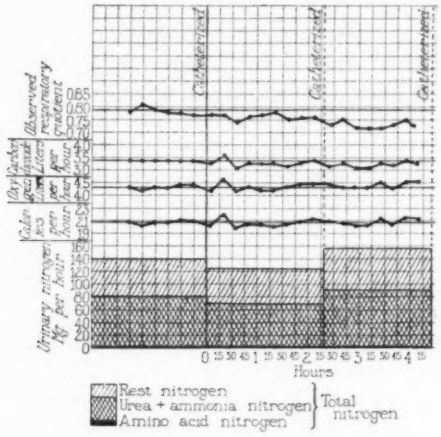

Fig. 1. Data of normal control experiment

tance; for example, a dog which has been used only for experiments over short periods of two or three hours' duration may not give constant results the first few times that it is employed in experiments of longer duration (six hours or more). It is often necessary gradually to accustom the animal to the longer periods before the desired constancy is obtainable. Other factors which are of equal importance include the type, quantity and time of feeding, the diet, and consequently the nutritional conditions of the animals. Experience has shown that in general the most constant results are obtained when the animals are maintained on an intermediate or low nutritional level and that the same animal may give relatively irregular results when changed to a high nutritional level. It has also been found that short-haired animals give much more constant and satisfactory results than the long-haired animals. In view of these facts it is obvious

that accurate and constant results can be obtained only by careful selection of animals and painstaking regard to training, diet, and other factors.

In the control experiments shown in figure 1 the results have been calculated as described; that is, the six tests obtained during the first two hours have been calculated on an average hourly basis and the total calories produced during the remaining period of approximately four hours have been compared with this "basal" value. The calculations have been carried out in this manner in order to make the results comparable with experiments in which amino acids or other substances were injected at the end of the basal period of two hours. In these five experiments on four different dogs it is seen that the difference is less than +2 calories for the total of four hour period in all experiments. The greatest difference (+1.8 calories) was obtained on the most perfectly trained animal in the group and was only an apparent difference, since the increase all occurred during the last one and one half hours when a violent wind storm made it necessary to change from the breathing of outside air to air taken from the small, closed gasometer room where one and often two observers were working. The experiment is valuable in again emphasizing the need of maintaining constant conditions. If we exclude the experiment just referred to, it is seen that the differences are all less than +2 per cent of the basal heat production over the same period of time and not more than 4 per cent in the case in which the elevation is obviously artificial. These results are particularly interesting in view of the statement frequently made that when the open-circuit gasometer method is employed, no importance should be attached to changes of less than 10 per cent. This is probably true in experiments in which the details referred to have been neglected, but with the methods and regimen employed in this laboratory we feel that variations of more than 2 per cent are of significance.

It is important to call attention to the fact that when the normal basal metabolism is determined continuously for periods of six hours, beginning approximately twenty-one hours after the last feeding, there is usually a slight progressive fall of the respiratory quotient. This is probably due to the fact that the period of fasting is becoming progressively longer. This observation is of significance when compared with the behavior of the respiratory quotient following injection of amino acids into normally nourished animals, in which the respiratory quotient shows a tendency to remain elevated or to rise above the basal value.

THE INFLUENCE OF THE RATE OF INJECTION OF THE SOLUTION OF AMINO ACID. In our previous work this factor has been controlled and held constant by arbitrarily adopting a period of ten minutes for injection as the standard, except in the experiments with phenylalanine (8) in which the larger volume of solution made it necessary to lengthen the period of injection. The question arose, however, as to whether it would be possible to

alter the specific dynamic action of alanine or glycine by injecting at a slow rate. The general plan of the experiments was to dissolve the required amount of amino acid in 50 cc. of water and perform experiments on the same animal in which the injection was given in the standard period of ten minutes and then in periods of time ranging from one hundred three minutes to one hundred twenty-nine minutes.

In the first three experiments, performed on two dogs, the specific dynamic action was greatly reduced by the slow rate of injection. The results seemed so clear-cut that we believed them to depend solely on the rate of injection. Further analysis, however, showed that the one animal on which two such results had been obtained, had been used eight to ten weeks before in experiments involving the giving of large amounts of carbohydrate (Karo syrup and cracker meal) in addition to the standard diet, combined with repeated injections of insulin. In the other animal no such factors were found. Following these experiments it was found by Wilhelmj and Mann (9), (10) that the specific dynamic action of the same amino acid in the same animal could be greatly altered by changing the nutritional status; that is, during total fasting, there was an increase of the specific dynamic action above the normal value obtained when the animal was receiving the standard diet; when total fasting was followed by the administration of diets high in carbohydrate, or even when the standard diet was augmented with carbohydrate following fasting, they found that the specific dynamic action was always reduced below the value obtained during total fasting and might, at times, be reduced below the value obtained when the animal was receiving the standard diet. These observations made it imperative to reinvestigate the effect of slow injections, using animals which had been on the standard diet with no modifications for sufficiently long periods to insure a balanced, stable nutritional condition, comparable to that existing in the standardized animals. Four additional animals were therefore used and in none of the experiments did we obtain a lowering or the specific dynamic action when the amino acids were injected very slowly; in fact, the total specific dynamic action was definitely higher in two of the animals. We do not believe that this higher value is of true metabolic significance, since these experiments are difficult to perform because of the difficulty in maintaining proper environmental conditions during the long period of injection, and slight movements and restlessness of the animal probably account for the higher value. In the remaining two animals the specific dynamic action was the same with the long and with the short periods of injection and we believe that these experiments represent the true conditions (fig. 3). In the experiment shown in figure 2 the total specific dynamic action following the injection over ten minutes amounted to 6.4 calories whereas, when one hundred twenty-two minutes were required for the injection, the total specific dynamic action was 10.1

calories. When these results are calculated as calories per millimol of amino acid deaminized, however, the values are practically identical, namely, 0.36 and 0.34 calorie, respectively. In other experiments the total specific dynamic actions are identical, that is, 12.2 and 12.0 calories

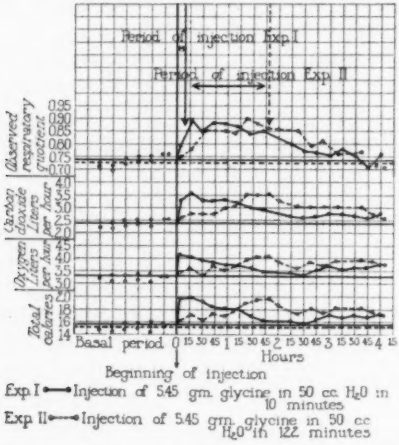

Fig. 2. The effects of rapid and slow administration of glycine

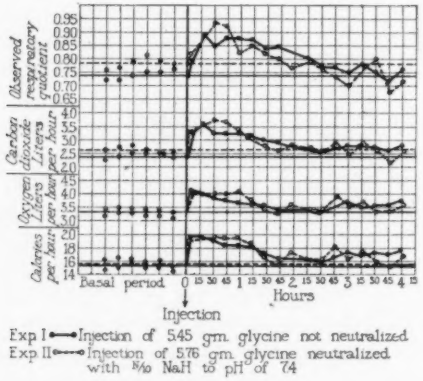

Fig. 3. The similarity of effect of administration of acid and neutralized solutions of glycine.

with the rapid and with the slow injections. The values per millimol of glycine deaminized do not agree as well as in the other experiment but are sufficiently alike to indicate that there is not an essential difference due to the rate of injection. When the slow and the rapid injections are compared

it is seen that with the slow rate of injection the increase in consumption of oxygen is gradual and reaches its peak in from one hour and fifteen minutes to one hour and forty-five minutes, whereas, with the rapid injection, the peak of oxygen consumption is reached either during the injection or within thirty minutes after the beginning.

Although we have not obtained entire agreement in this series of experiments, we feel reasonably justified in concluding that the specific dynamic action is practically uninfluenced by altering the rate of injection between the usual period of ten minutes and that of two hours or more, provided that the animals are in the standard dietary condition usually maintained.

THE INFLUENCE OF THE HYDROGEN-ION CONCENTRATION OF THE SOLUTION OF AMINO ACID. The next point to be investigated was the effect of altering the hydrogen-ion concentration of the solution of amino acid in an attempt to determine whether or not the carboxyl group of the amino acid, in virtue of its acidic properties, contributed to the increase in the production of heat and the elevation of the respiratory quotient which follow the intravenous injection of unneutralized solutions of amino acid. The experiments were performed by injecting the same amino acid when dissolved in 50 cc. of distilled water and comparing the response in the same dog when the solution of amino acid either was neutralized to pH 7.4 or was made definitely alkaline with sodium hydroxide. In figure 3 two such experiments are shown. In experiment 1 the amino acid was simply dissolved in 50 cc. of distilled water, whereas in experiment 2 the solution was neutralized to pH 7.4 with sodium hydroxide. In general there is relatively little difference between the two curves; the total specific dynamic actions are practically identical (6.4 and 6.5 calories). The nonprotein respiratory quotient for the total period of four hours after injection is 0.81 with the unneutralized solution and 0.77 when the solution was neutralized to pH 7.4; this difference is so slight that its significance is doubtful. When the curves showing the individual determination of the respiratory quotients are examined it is seen that there is an abrupt rise in both experiments, following which both curves slowly decline. In experiment 2, with the neutralized solution, the quotient tends to drop below the original basal values; this is not so prominent in experiment 1, with the unneutralized solution. Due to the higher basal quotient in experiment 2, this difference is more apparent than real and the curves for production of carbon dioxide show that there is relatively little retention of carbon dioxide. It should be emphasized that even when the solution of amino acid was neutralized to pH 7.4, there was a definite elevation of the respiratory quotient above the basal level, which persisted for approximately two hours, and that following this there was little evidence of retention of carbon dioxide during the next two hours. These facts are in accordance with our previous observations and suggest that the elevation of the respiratory quotient which

nearly always follows the injection of an amino acid in normally nourished animals is of true metabolic significance and is not due merely to an acid-base shift dependent on the carboxyl group of the amino acid.

The results of a somewhat different type of experiment on another dog are as follows: In the first experiment we injected 8.18 grams of glycine dissolved in 50 cc. of water; in the second experiment, 8.18 grams of glycine was dissolved in 100 cc. of 0.495 normal sodium hydroxide, the resultant solution being strongly alkaline to phenolphthalein. There was an immediate fall in the respiratory quotient to 0.58 during the injection and to 0.49 immediately after completion of the injection; following these tests the quotient rose to a value approximating the basal level. In this experiment we have definite evidence of retention of carbon dioxide as a result of the alkali injected. This experiment has been calculated in three different ways: 1, without correcting for the carbon dioxide which was retained, the figures being used as actually obtained; 2, assuming that the true, observed respiratory quotient for the total period of four hours after injection was 0.816; that is, the same as in the experiment in which the glycine was given in distilled water, the oxygen was multiplied by 0.816, and the resulting figure was assumed to represent the true value for production of carbon dioxide; and 3, calculating that 1.98 gram of sodium hydroxide was injected in the 100 cc. of 0.495 normal solution and that this would cause the retention of 0.555 liter of carbon dioxide, this latter figure was then added to the observed production of carbon dioxide. It is seen that in general the results are similar with the use of these three methods of calculation; the resulting values for the total sepcific dynamic action are, respectively, 14.9, 16.1, and 15.6 calories, which are all close to the value of 16.0 calories obtained in the experiment in which the glycine was injected in distilled water.

These experiments show clearly that the usual rise in the respiratory quotient which follows the injection of non-neutralized solution of glycine is not dependent on the liberation of carbon dioxide resulting from the acidic properties of the carboxyl group, and that the specific dynamic action is unaltered even when glycine is injected in alkaline solution. Many similar experiments were performed with animals on high carbohydrate diets, and during fasting, and they all yielded results which were in agreement with these conclusions.

THE INFLUENCE OF THE OSMOTIC PRESSURE OF THE SOLUTION OF AMINO ACID. In attempting to evaluate this factor we encountered certain difficulties based primarily on the necessity of comparing metabolizable and nonmetabolizable substances and in attempting to differentiate specific and nonspecific effects. When solutions of amino acid or glucose are injected, certain metabolic processes are immediately set in motion involving deaminization, storage or oxidation, and so forth, so that the osmotic

TABLE 1

A study of the influence of the osmotic pressure of various solutions when compared with the amino acid solution, dog 1

BASAL PERIOD (PER HOUR)					AFTER INJECTION					SPECIFIC DYNAMIC ACTION OR INCREASE OVER BASAL CALORIES	INCREASE OVER BASAL CALORIES	LENGTH OF EXPERIMENT AFTER INJECTION	COMMENT
Total oxygen	Total carbon dioxide	Protein nitrogen	Nonprotein respiratory quotient	Total calories	Total oxygen	Total carbon dioxide	Protein nitrogen	Nonprotein respiratory quotient	Total calories				
liters	*liters*	*gram*			*liters*	*liters*	*gram*				*per cent*	*hours*	
4.089	3.237	0.260	0.78	19.1	20.147	16.446	0.716	0.79	94.9	16.0	20.3	4.13	8.18 gm. glycine in 50 cc. of water; calories of specific dynamic action per millimole of glycine deaminized = 0.27, 9–4–29, 15.7 kgm.
4.153	3.098	0.128	0.73	19.4	18.044	13.950	0.755*	0.76	84.5	6.9	8.9	4.00	8.18 gm. sodium chloride in 50 cc. of water, 9–25–29, 15.6 kgm.
4.282	3.219	0.093	0.74	20.1	18.610	14.025	0.435*	0.75	87.5	5.5	6.7	4.08	3.20 gm. sodium chloride in 50 cc. of water, approximately isotonic with 8.18 gm. glycine, 10–17–29, 15.5 kgm.
4.356	3.279	0.079	0.75	20.5	17.862	14.345	0.369*	0.80	85.1	2.5	3.0	4.03	17 gm. glucose in 50 cc. of water; approximately isotonic with 8.18 gm. glycine, 10–10–29, 15.4 kgm.
4.323	3.155	0.198	0.70	20.0	18.079	12.952	0.878	0.68	83.7	1.7	2.1	4.10	0.450 gm. sodium chloride in 50 cc. of water, 12–18–29, 16.9 kgm.
4.198	3.176	0.153	0.74	19.7	17.005	12.318	0.597	0.70	79.0	+0.8	+1.0		Normal metabolism determined for approximately 6 hours (3.97 hours after first 2 hours), 12–2–29, 16.5 kgm.
4.324	3.394	0.140	0.78	20.4	18.289	13.555	0.613	0.73	85.4	+0.3	+0.35		Normal metabolism determined for approximately 6 hours (4.17 hours after first 2 hours), 12–20–29, 17.0 kgm.

* Total nitrogen excreted during entire period after injection, minus the "extra" urea and amino acid nitrogen which presumably resulted from the "washing out" process accompanying the diuresis.

pressure of the original solution is quickly altered and the changes dependent on the osmotic pressure, if any, are overshadowed by the specific changes in metabolism. Comparison of the results obtained under these circumstances with those which follow the injection of some totally inert substance such as sodium chloride, which is probably unaltered by the cells and eliminated from the body as soon as possible, is quite likely to be not entirely justified. Nevertheless we have carried out the following series of experiments on each of two dogs: 1, the injection of the amino acid; 2, the injection of the same quantity of sodium chloride in the same volume of solutions; 3, the injection of a quantity of sodium chloride which was approximately isotonic with the amino acid solution; and 4, the injection of a solution of glucose approximately isotonic with the solution of amino acid. The results obtained with the two animals were practically the same and the results obtained in one of them are shown in table 1. For the sake of comparison we have included in this table the results obtained on this animal when injections were not made, and the normal metabolism was determined for approximately six hours with catheterization every two hours. The injection of 8.18 grams of glycine in 50 cc. of water resulted in a total specific dynamic action of 16.0 calories or 0.27 calorie per millimol of amino acid deaminized. The total increase in metabolism (16.0 calories) over a period of 4.13 hours is equivalent to an increase of 20.3 per cent above the basal metabolism over a similar period. In the second experiment we administered 8.18 grams of sodium chloride in 50 cc. of water and obtained an increase of 6.9 calories or 8.9 per cent. The increase in consumption of oxygen which resulted from the latter injection was definite and was maintained as a smooth curve during the entire period of four hours after injection. In the next experiment we injected 3.20 grams of sodium chloride in 50 cc. of water, which was approximately isotonic[2] with the solution of amino acid. Following this there was a somewhat irregular elevation in consumption of oxygen which also persisted during the entire period; the calculated increase in production of heat amounted to 5.5 calories, or 6.7 per cent. As a further control on these results, another experiment was performed in which 50 cc. of physiologic sodium chloride solution was injected. The resulting change in consumption of oxygen was so slight that it was practically within the range of the normal fluctuations in metabolism over a similar period and when expressed in terms of production of heat the result was an increase of 1.7 calorie or 2.1 per cent. This result confirms previous unpublished reports of experiments which have shown physiologic sodium chloride solution to be without appreciable effect. The results with the concentrated saline solutions show clearly that the increase in consumption of

[2] The isotonicity of these solutions is only approximately the same.

oxygen is not directly proportional to the tonicity of the solution, since approximately the same increase was secured with 50 cc. of a 16 per cent solution as with 50 cc. of a 6 per cent solution.

On the basis of these experiments alone it would possibly be justified to conclude that from 30 to 50 per cent of the specific dynamic action of the solutions of amino acid was of a nonspecific nature, resulting from the effects of osmotic pressure. In order to test this hypothesis, experiments were performed using solutions of glucose which were approximately isotonic with the solutions of amino acid. We had previously noted that the specific dynamic action of glucose, unlike that of the amino acids, is characterized by considerable irregularity even in the same animal kept under as nearly constant conditions as possible. We have attributed this irregularity to differences in the mode of utilization of the glucose. For example, the increase in production of heat would be very small if most of the injected glucose were stored but would be correspondingly greater when a considerable portion was combusted. Even when the injected glucose is largely stored, however, the osmotic pressure effect of the solution should exert its influence, and we were interested in comparing the specific dynamic action under these conditions with the results obtained with approximately isotonic saline and amino acid solutions. If the osmotic pressure effect could be predicted from the experiments with saline, then we should expect the glucose to produce an elevation in production of heat of approximately 6 or 7 calories. Actual experiment, however, gave an increase of only 2.5 calories. Practically all of this elevation occurred in the first hour, and corresponded to the time of elevation of the respiratory quotient. Following this, both the production of heat and the respiratory quotient returned approximately to the basal level.

From these results it is clear that the osmotic pressure effect is entirely overshadowed by the specific metabolic changes induced by the material injected. Under the next heading it will be shown that the specific dynamic action of alanine and of glycine are the same whether the amino acid be given intravenously or by mouth, which permits the conclusion that if effects of osmotic pressure do contribute a certain fraction of the elevation in metabolism resulting from administration of amino acid, then this effect is also a factor following oral administration.

Lusk (4) found that oral administration of 6.7 grams of sodium chloride in 150 cc. of water to a dog weighing 8.7 kgm. caused no elevation in metabolism in spite of the fact that large quantities of the sodium chloride were absorbed and excreted in the urine. The difference between his results and ours may possibly be due to the fact that the osmotic pressure of the orally administered solution is readjusted in the intestinal canal and is absorbed as a practically isotonic solution, which, as we have shown, may cause practically no change, or at least only an insignificant elevation in

production of heat when given intravenously. These considerations bring up an interesting point which possibly would explain the elevation in production of heat which follows the intravenous administration of hypertonic saline solutions; namely, that when a hypertonic solution enters the blood stream, attempts are immediately made to render the solution isotonic. In this process, water is rapidly withdrawn from the tissues, and it is possible that this tissue fluid may bring with it certain stores of carbohydrate, protein or amino acid, and even fat; the entrance of these stored food materials into the blood stream may set up reactions leading to their utilization, with a resultant increase in basal production of heat which in reality is the specific dynamic action of these food materials.

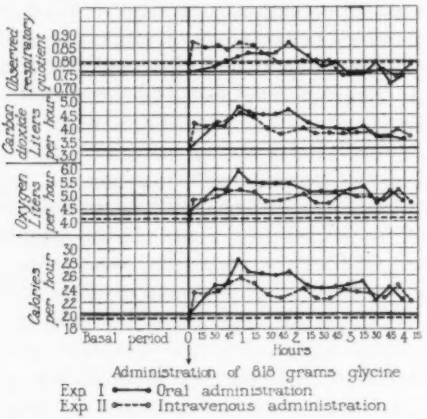

Fig. 4. The effects of intravenous and oral administration of glycine

A COMPARISON OF THE SPECIFIC DYNAMIC ACTION OF AMINO ACIDS ADMINISTERED INTRAVENOUSLY AND BY MOUTH. As we have stated, the primary purpose of the experiments just described was to assist in determining whether or not the response to amino acids administered intravenously could be considered strictly comparable to the response obtained by administration orally. From the data obtained in the foregoing experiments, we would anticipate that intravenous and oral administration should give practically identical results, and the present experiments confirm this deduction. In the experiments shown in figure 4, 8.18 grams of glycine, dissolved in 50 cc. of water, were given intravenously, and later by mouth. The response in the two instances was almost identical. The total specific dynamic action following intravenous administration was 16.0 calories, and by oral administration 16.2 calories; per millimol of glycine deaminized, the specific dynamic actions were 0.27 and 0.22 calorie following, respectively, intravenous and oral administration. The respective

respiratory quotients for the total period of four hours after administration were 0.79 and 0.78. In another experiment 5.83 grams of glycine was given intravenously on two different occasions and the response was compared with that obtained when 10 grams was given orally. In the two experiments with intravenous administration, the total specific dynamic action was 8.8 and 10.8 calories, respectively, and for the purpose of comparison we will consider the average of these, or 9.8 calories. The ratio of the quantity of glycine given intravenously to that given orally was 1 to 1.71, whereas the ratio of the respective total specific dynamic actions (9.8 to 16.3) was 1 to 1.66. The ratio of the average quantity of extra urea nitrogen (glycine deaminized), in the two experiments in intravenous administration, to the extra urea nitrogen obtained in the experiment with oral administration is 1 to 1.79 (0.573 to 1.03). The agreement in these ratios is sufficiently good to consider them as being identical. The specific dynamic actions per millimol of glycine deaminized are likewise practically identical.

The results of these experiments are definite, and prove conclusively that the quantitative response is the same whether the amino acids be administered intravenously or orally and are in perfect agreement with the results previously reported by Weiss and Rapport and later by Nord and Deuel.

SUMMARY

The basal production of heat in normal well trained dogs which are in a balanced, stable, nutritional condition is constant when it is determined almost continuously for periods of approximately six hours, provided that environmental conditions are maintained constant. When the last four hours of the period of six hours are compared with the first two hours (considering the latter as the basal value), the difference should not exceed about +2 per cent. It must be clearly understood, however, that this constancy depends on careful selection and training of animals and the maintenance of standard nutritional conditions. During such a period of six hours of observation, there is usually a progressive fall in the respiratory quotient dependent on the increasing length of the period of fasting.

The influence of the rate of injection of the solutions of amino acid was studied in six animals. The period of injection varied from the usual period of ten minutes up to one hundred twenty-nine minutes. In three experiments on two animals the slow rate of injection caused marked lowering of the total specific dynamic action. In one of these animals, previous experiments which involved radical alterations in the diet may have been responsible for the result obtained. Because of these complications, the experiments were repeated on four animals which had been receiving the standard diet, without alteration, for sufficiently long periods

to establish nutritional equilibrium. In these four animals the total specific dynamic action was not reduced by the slow rate of injection; in fact, in two of the experiments, it was higher with the slow injection. No importance is attached to the higher value obtained since it is probably due to restlessness on the part of the animal resulting from the long period of injection, during which time it is also difficult to maintain constant environmental conditions. In two of the animals, the specific dynamic action was the same with the slow and with rapid injections and it is probable that these experiments represent the true conditions; namely, that the specific dynamic action of a given quantity of alanine or glycine is independent of the rate of injection within the limits of time ranging from ten minutes to approximately two hours.

The influence of the hydrogen-ion concentration of the solution of amino acid was studied by the following experiments: 1, injection of the amino acid dissolved in distilled water; 2, injection of a solution neutralized to pH 7.4, and 3, injection of a solution of amino acid made definitely alkaline with sodium hydroxide. The results show clearly that the specific dynamic action is not influenced by the reaction of the solution injected and that the rise in the respiratory quotient which usually follows the injection of alanine or glycine into normally nourished animals is not dependent on the acidic properties of the carboxyl group.

The injection of saline solutions, approximately isotonic with the solution of amino acid, was found to produce an appreciable elevation of the production of heat, which in these experiments amounted to from 30 to 50 per cent of the specific dynamic action of the amino acid. Physiologic sodium chloride solutions were without appreciable effect. It is likely that the use of a substance which is not utilized by the organism is not a fair test of the effect of osmotic pressure of the solutions of amino acid, and this is borne out by the results obtained when solutions of glucose of approximately the same tonicity were injected, in which case the specific dynamic action was considerably less than the elevation in production of heat caused by an approximately isotonic solution of saline.

The specific dynamic action of alanine or glycine is the same whether the amino acids be given intravenously or by mouth.

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PHYSIOLOGIC RESPONSES AND IMMUNE REACTIONS TO EXTRACTS OF CERTAIN INTESTINAL PARASITES

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Investigations on the physiologic action of the venom of the rattlesnake and the honeybee aroused our interest in the pharmacology of parasitic extracts. A few preliminary experiments indicated that such extracts are similar, in their physiologic manifestations, to the venom of the rattlesnake and the honeybee. In our first experiments we used saline extracts of fresh *Ascaris lumbricoides*, a round worm from swine. When given intravenously to dogs the extracts of this worm produced an immediate profound fall in blood pressure. When needled into the skin of a human being, whealing and an arteriolar flare resulted. This was also the case with extracts of *Strongylus equinus*. Similar experiments with extracts of the larvae of the insect *Gastrophilus intestinalis*, recovered from the intestinal tract of the horse, were negative. Extracts of the common earthworm *Lumbricus terrestris* were also negative. While these experiments were in progress we also investigated the physiologic response of dogs to extracts of the intestinal parasites *Taenia pisiformis* and *Toxocara canis*, with which dogs are commonly infested. The results of these experiments promised to shed some light on the physiology of host-parasite relationship. Consequently we devoted our further attention exclusively to these common parasites of the dog.

Many investigations have been made with extracts of parasitic worms. Comprehensive reviews have been published on the subject by Phisalix (1922), by Faust (1924) and by Taliaferro (1929). Three reports bearing on this subject should be mentioned here. A most extensive investigation was made by Shimamura and Fujii (1917) on extracts of *Ascaris lumbricoides* obtained from swine. These workers isolated from the body fluids and the powdered body of the worm an "albumose-peptone" which they designated as "askaron." This principle which was very active in small doses, produced all the toxic symptoms present in ascariasis when injections of watery extracts were made. According to these workers, askaron is widely distributed among round worms; it was present in all species analyzed by them. These workers reported the significant observation that

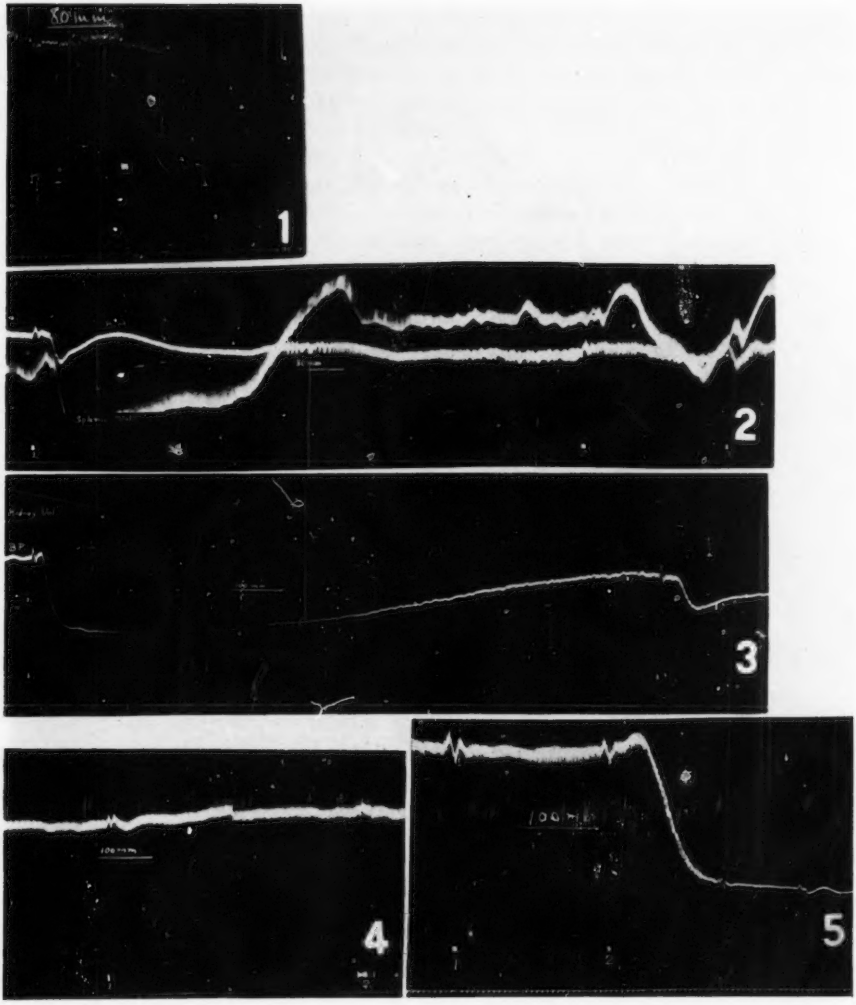
following the injection of a toxic dose of askaron there immediately resulted a high degree of resistance toward a second injection of as many as twenty-five lethal doses. This resistance reached its height in twenty-four hours and slowly disappeared; it was gone in about two months. They found that a horse could be immunized by repeated injections, until it could withstand 400 lethal doses. The serum of such an immunized horse (1 cc.) afforded no protection against askaron when injected into guinea pigs, from which they concluded that the immunity must be essentially cellular. The authors pointed out that although many of the symptoms of intoxication by askaron resemble anaphylaxis, the substance could not be considered an anaphylactogen, since it is primarily toxic. The experiments of these workers were confined almost entirely to the symptoms manifested by animals poisoned by askaron.

Van Es (1917) investigated the reaction of the blood pressure of horses to extracts of larvae of *Gastrophilus intestinalis*. He found that horses which harbored the larvae were thrown into severe anaphylactic shock by injections, whereas weaned colts which never had been infested were unaffected by extracts of the larvae. Van Es also investigated the effect on dogs of extracts of various helminths of canines.

Emery and Herrick (1929) described the physiologic effects of extracts of *Ascaris lumbricoides* when injected into dogs. They found that the extract was markedly a depressor; the fall in blood pressure was accompanied by diminished volume of the splanchnic viscera and by increased volume of the hind limb. These observations suggested to them that the extract was possibly related to histamine. As a result of certain experiments with the perfused intestine of rabbits they concluded, however, that the effects were not due to histamine.

In our study we are reporting the physiologic alterations produced by the intravenous injection into the dog of saline extracts made principally from two intestinal parasites of the dog. At the outset we were interested in determining whether the action of such extracts resembled that of rattlesnake venom and bee venom. These experiments indicated in general a close resemblance, but certain fundamental differences are brought out in this communication.

Methods. Most of our experiments were performed with the dog tapeworm, *Taenia pisiformis*. This is the common parasite of the dog in the region where our experiments are conducted. In brief, the life history is as follows: The eggs of the parasite pass out with the feces of the dog, and, when eaten by a rabbit, produce in the latter the bladder worm stage known as *Cysticercus pisiformis*, which, when ingested by a dog develops into the adult form of the tapeworm. Infection from dog to dog is thus impossible without the intervention of rabbits. It is possible to predict that when dogs are reared under conditions in which they do not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have access to rabbits, infestation with *Taenia pisiformis* will not occur. This point is important since it is impossible to know whether an uninfested adult dog never has been infested, or whether an infestation has terminated spontaneously. The procedure is not so simple in the case of the round worm, *Toxocara canis*, with which we are also concerned. The life cycle of this parasite is direct. Thus it can be transmitted from dog to dog, and pups are known to be decidedly susceptible to infestation, whereas old dogs are refractive. In order to secure dogs that never have been infested, pups must be taken at birth and reared under the most rigorously controlled conditions.

The extracts used were prepared in the following manner: The worms were collected immediately after the death of the animal, or removed surgically during life. They were cleansed in tap water, and cut into fine pieces which were spread out in a single layer over the bottom of Petri dishes. These dishes were then placed for twenty-four hours in an oven kept at 37.5°C. The particles were then triturated in a mortar to a coarse powder, and afterward were further reduced by being shaken with pyrex beads on an automatic shaker. The finely powdered material was placed over a calcium chloride desiccator. In each experiment, a standard dose of 8 mgm. of the dried powder for each kilogram of body weight was used. It was treated as follows: the requisite dose was ground up with normal saline in the proportion of 8 mgm. for each cubic centimeter, and centrifuged; the supernatant fluid only was employed for injection.

RESULTS. When *Taenia pisiformis* extract was given intravenously in a dose of 8 mgm. for each kilogram of body weight, there resulted a profound and rapid fall in blood pressure usually with slow recovery, so that in one half hour to one hour the blood pressure had returned to about the normal level. Occasionally the standard dose was lethal. The depressor

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Fig. 1. The lethal effect on the blood pressure, of administration of 8 mgm. for each kilogram of body weight, of *Taenia pisiformis* extract. Zero base line is indicated by the signal marker. The time marker registers intervals of five seconds. The same holds true for all subsequent figures.

Fig. 2. Behavior of splenic volume during the fall in blood pressure produced by extract of *Taenia pisiformis*. The standard dose was injected at signal *1* and repeated at signal *2*.

Fig. 3. Behavior of renal volume accompanying the depressor activity of an extract of *Taenia pisiformis*.

Fig. 4. The immunity of a heavily infested animal to four times the usual depressor dose of extract of *Taenia pisiformis*. The animal was shown to be heavily infested by opening the jejunum.

Fig. 5. Blood pressure of etherized dog. At *1*, a saline extract made from earth worms was injected (16 mgm. for each kilogram of body weight). At *2*, saline extract from the dog round worm, *Toxocara canis* was injected (4 mgm. for each kilogram of body weight).

effect occurred after a latent period of thirty to sixty seconds (fig. 1). Coincident with the fall in blood pressure there was decrease in the volume of the spleen and kidney (figs. 2 and 3). When the blood pressure had returned to near the normal level, a subsequent injection of the same or a larger dose did not, with one or two exceptions, produce further effect on the blood pressure. To determine the duration of this refractory condition, which was demonstrated repeatedly, a dog was given an injection of the standard dose, and the blood pressure in his femoral artery was recorded. Five days later, the experiment was repeated, except that the opposite femoral artery was used for cannulation. Injection of the same dose did not produce a fall in blood pressure. Twelve days later, this dog was again given an injection, with the same negative result. This observation raises the question as to the nature of the resistance to a second injection. In an effort to shed light on this problem, we injected the standard dose of *Taenia pisiformis* extract into four dogs, known to be heavily infested with this parasite. In each of these cases the injection was not followed by a depressor response (fig. 4). In one instance, five times the standard dose was given without any fall in blood pressure. Several animals gave very little evidence of fall in blood pressure, and at necropsy were found to be infested. Dogs that never had been infested invariably responded by decided fall in blood pressure. We, therefore, suspect that when the blood pressure fails to fall the animal is either heavily infested (which can readily be detected) or has developed immunity in the course of past infestation.

The reactions to extracts of *Toxocara canis* were similar, with the striking difference, however, that a second injection, either immediately after the first, or a week later, was still effective in lowering the blood pressure. There followed a fall in blood pressure after each injection, accompanied by lessened volume of the kidney and spleen, and increased volume of the hind limb. Although the depressor reaction from the extracts of the two worms appeared to be similar, the toxic principle was distinct, as shown by the fact that when a dog did not respond to an extract of *Taenia pisiformis*, injection of the extract of *Toxocara canis* produced a depressor response.

COMMENT. The physiologic action of the parasitic extracts differs from that of rattlesnake venom and bee venom in a number of important particulars. The swelling of blood cells and hemolysis produced by the latter, in vivo and in vitro, does not occur when parasitic extracts are added to the blood in vivo or in vitro. Diffuse hemorrhage, which is a prominent feature of rattlesnake and bee venom poisoning, does not follow injections of parasitic extracts.

It is of interest that helminths contain a toxic principle, the vasomotor effects of which are strikingly similar to those of rattlesnake venom and bee venom. What its function can be is difficult to determine. Extracts of the common earthworm have no effect on the blood pressure of the dog, nor have

extracts of larvae of *Gastrophilus intestinalis*. That the depressor principle has a function in protecting the worm against the host is improbable since the last named organism lacks this depressant. The work of Shimamura and Fujii (1917) and of Emery and Herrick (1929) and our data on *Toxocara canis* may be taken as evidence for the existence of a toxic principle, with similar properties, that is universally distributed through the nematode class. As to whether or not our results with *Taenia pisiformis* apply to other Cestoda, we have not enough data to enable us definitely to state.

The toxic effect of parasitic extracts, which has been described by many workers, frequently has been interpreted as an anaphylactic response. However, since extracts of *Taenia pisiformis*, injected into dogs that never have been infested, produce severe reactions, and injections into heavily infested dogs produce negative results, it is obvious that the reaction is not one of anaphylaxis. As commonly understood, the first dose of the antigen employed for sensitizing animals is innoxious which is not true of the extracts under consideration.

It would appear, from these experiments, that the parasites harbored by dogs elaborate substances against which the host develops immunity, and that when immunity has developed, the dog remains infested but subsequently is not greatly injured by the parasites. A comparable condition exists in the case of diphtheria and typhoid carriers. Such carriers continue to harbor the organisms even though they possess immunity to their toxins. It is evident that a heavy infestation with cestodes or other helminths should be more disastrous in young dogs that have not developed immunity to their toxins.

A number of reasons exist for the belief that the toxic principle is not histamine: it is destroyed by boiling, and repeated injection of the substance is quickly followed by a condition of extreme tolerance which appears to be immunity. Moreover, it lowers the blood pressure of rabbits, which is entirely different from the action of histamine on this animal. Indeed we have evidence that there is no histamine present in the parasitic extracts as prepared by us, since alcoholic extraction of finely ground *Strongylus equinus* did not yield a depressor substance.

The experiments give promise of supplying a method of investigating the broad problem of immunity to infestation by helminths. It may be predicted that when a dog is heavily infested it is immune to the depressor effect of extracts made from the parasite. Our experiments to date have given no direct proof that the depressor constituent is the antigen which is directly responsible for the development of the immune state. To prove this it would first be necessary to obtain the depressor constituent in a reasonably pure form.

Another question raised by these researches is concerned with the origin

of the immunity or refractoriness which develops so rapidly when a dog is given injections of an extract of *Taenia pisiformis*, but our investigations have thus far failed to reveal any substantial evidence on this point.

SUMMARY

A study was made of extracts of various parasitic worms with regard to their effect on blood pressure. The vasomotor action was comparable to that of bee venom and rattlesnake venom.

Taenia pisiformis extracts which were markedly depressor in dogs not previously infested had no effect on the blood pressure of dogs that were heavily infested. Thus the dog is apparently able to develop immunity to the toxic substances elaborated by the parasite.

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CHANGES IN THE BASAL METABOLIC RATE ACCOMPANYING THE CONDITIONED STATE INDUCED BY MORPHINE

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Subcutaneous injection of morphine into dogs produces in a few minutes salivation, retching and vomiting. After seven or eight daily administrations, Collins and Tatum (1925) found that the animals would salivate and sometimes vomit before the drug was given. The conditions immediately preceding the injection such as the sight of the experimenter or of the hypodermic needle called forth the reaction. The salivary secretion occurring during this reflex was quantitatively studied by Kleitman and Crisler (1927), who associated the injection of morphine with a certain definite set of conditions which could be maintained for an hour or more before the injection. It was found that after a few daily injections of morphine, salivation occurred before the injection in increasing amounts from day to day until a certain level was reached. Accompanying this conditioned salivation, it was also observed that panting, whining and occasionally vomiting occurred. The symptom complex of which these objective signs are a part was called nausea by the writers.

This state of nausea resembles very markedly some of the responses classed as emotional or excitement reactions by Cannon and Britton (1926). Cats when restrained in a holder showed uneasy twitching of the tail, occasional mewing and turning of the head, minor signs of excitement, the authors state. The studies presented in this paper are an attempt to determine whether the conditioned state, induced by morphine, is accompanied by an increase in metabolism over the basal metabolic rate.

It is asserted that minor signs of excitement in the cat as well as strong emotion are accompanied by increased adrenal secretion (Cannon and Britton, 1926). It has also been shown that intravenous injection of adrenalin in doses claimed to be within a physiological range, lead to increases over the basal metabolism (Boothby and Sandiford, 1923). Therefore, if increases over the basal metabolism occurred during the conditioned morphine excitement, it seemed important to determine whether increased adrenal secretion was an important factor in producing the augmented metabolism.

METHOD. 1. *Apparatus.* The Benedict portable respiratory apparatus

was used with the mask for covering the dog's muzzle as described by Kunde (1923) with the addition of a small outlet on its under surface connected by rubber tubing with an S-shaped glass tube containing water in the bend of the tube. Saliva could flow off by way of this tube while air could not enter the system. The joint between nose and mask was tightened by fastening a rubber band (about two inches wide) over the line of contact (Kunde et al., 1930). A graphic record of the oxygen consumption and respiration was made by a writing point fastened to the spirometer bell recording on a smoked drum. The apparatus was tested for leaks up to the point of junction of the mask with the dog by placing a 5 gram weight on the spirometer bell, placing a small glass bottle in the opening of the rubber dam, and opening the valve so that the mixture of oxygen and air in the system circulated through the mask being forced through the circuit by the rotary blower. If the writing point on the bell did not move during five minutes, the machine was judged to have no leak. Duplicate basal metabolism tests with a small weight on the spirometer bell were also made to test for leaks.

2. *Food and care of dogs.* Five dogs were used, each weighing about 10 kilograms. They were dewormed and kept dry and free from mange. Each dog was fed at least 20 hours before a test with a standard diet of 200 grams of meat, 250 cc. milk, and from 100 to 200 grams white bread, the amount of bread being determined by what the animal would eat regularly day after day without leaving any. This amount would usually be less in warm weather than in cold weather. Two to three tablespoons of bone meal were put into the mixture. Each dog was allowed to run about alone in an open pen or room each day and defecation permitted before being brought to the test room.

3. *Procedure.* Daily determinations of metabolism were made until the average metabolism for a two-week period lay within the range found by Kunde and Steinhaus (1926) in their study of basal metabolism in normal dogs. For Doctor Kunde's dogs, the average basal metabolisms ranged from 686.0 to 843.0 cals. per square meter per 24 hours, the average value being 777.5 cals. per square meter per 24 hours. It would often require weeks of training before this basal level was reached. Each day the dog was weighed, brought to the metabolism room, and rested for forty-five minutes on a table. Then a fifteen minute test was made with the room quiet. At the close of the test pulse rate and rectal temperature were recorded. The animal was then taken to his cage and fed, the next test being made at least 20 hours after feeding.

Following the two week control period, the conditioned reflex was established in the following manner. A metabolism determination was made every other day, and immediately at the close of each test, while the dog's muzzle was still in the mask, an injection of $\frac{1}{2}$ grain of morphine sulphate

was given subcutaneously. The dog was held quietly on the table. Three or four minutes later the dog would usually attempt to draw his head from the mask, and would retch, or vomit mucus. The morphine stupor then followed. The dog's head was then removed from the mask, and he was taken to his cage. The dog was given food immediately, but usually would not eat for several hours. To prevent the direct effect of food or of morphine on the next metabolism test, the dog was not given a metabolism test next day. He was fed early the day after an injection and 20 hours later was taken to the metabolism room for a metabolism test immediately followed by injection of morphine.

After about three of these tests followed by morphine, the dogs would often struggle against having the mask put on, and would salivate somewhat during the test. After a few tests the animal would remain fairly quiet on the table if the experimenter held the dog's legs and head gently in place, a procedure which had no effect when tried during the control determinations of basal metabolism. These determinations of metabolism followed by morphine injections were made every other day for a month. After an interval in which the metabolism returned to the basal level, another control period was taken, followed by another period of morphine tests. It was thought that possibly an excess oxygen consumption during the morphine test period might be due to swallowing of saliva containing air. For this reason, the salivary glands (sub-maxillary, sublingual, parotid and orbital) were removed from two dogs already tested and a control period and a morphine period repeated on them.

Denervation of the adrenal glands was performed next on two new dogs and one dog which had been given three control basal metabolism periods and three morphine test periods. The method of denervation of the adrenals is similar to that outlined by Cannon (1919). The right adrenal gland was removed and also the left lumbar sympathetic chain between the diaphragm and the kidney. Next the thoracic sympathetic chain on the left side was removed for about two inches just above the diaphragm, an incision being made for this purpose between the 10th and 11th ribs.

The animal was then allowed to recover, but was brought to the testing room each day and put through the routine of rest and metabolism testing. After two weeks of control basal metabolism tests, the injections of morphine were made every other day as in the previous experiments. Two other dogs were subjected to the denervation operation and were killed after five weeks for histological examination of the region where the sympathetic chain had been removed to see if regeneration had occurred.

Results. A. In the five dogs studied, the average of the first morphine test period showed the following percentage increase over the average of the preceding basal metabolism control period; unoperated dogs, A, 23.7 per cent; B, 28.0 per cent; C, 13.0 per cent: adrenal operated dogs, D, 22.2

TABLE 1

Summary of control basal metabolism tests and metabolism tests during morphine conditioning periods

DOG	PREVIOUS TREATMENT	SERIES	PERIOD	NUMBER OF TESTS	AVERAGE WEIGHT	AVERAGE CALORIES PER 24 HOURS	AVERAGE CALORIES PER SQUARE METER PER 24 HOURS	TEST AVERAGE MINUS CONTROL AVERAGE PER 24 HOURS	PER CENT INCREASE OF TEST PERIOD AVERAGE OVER CONTROL PERIOD AVERAGE	P
					kgm.			*calories*		
A	Normal	I	Control	14	8.6	368.79	782			
	Normal	I	Test	14		456.11		87.32	23.7	0.01−
	Normal	II	Control	14	9.4	366.32	730			
	Normal	II	Test	10		428.69		62.37		0.01−
	Salivary glands removed	III	Control	17	8.9	372.35	775			
		III	Test	9		440.43		68.08		0.01−
	Right adrenal gland removed and left adrenal gland denervated	IV	Control	15	8.5	370.38	795			
		IV	Test	13		418.15		47.77		0.01−
B	Normal	I	Control	14	11.6	434.42	750			
	Normal	I	Test	10		556.03		121.61	28.0	0.01−
	Normal	II	Control	14	12.7	447.46	730			
	Normal	II	Test	17		444.75		−2.71		0.7+
	Salivary glands removed	III	Control	15	11.8	447.03	750			
		III	Test	10		438.64		−8.39		0.2−
C	Normal	I	Control	25	10.3	434.32	820			
	Normal	I	Test	10		490.71		56.39	13.0	0.01−
D	Right adrenal gland removed and left adrenal gland denervated	I	Control	13	12.3	494.34	825			
		I	Test	13		604.12		109.78	22.2	0.01−
E	Right adrenal gland removed and left adrenal gland denervated	I	Control	15	8.6	333.8	710			
		I	Test	10		446.1		112.3	33.6	0.01−

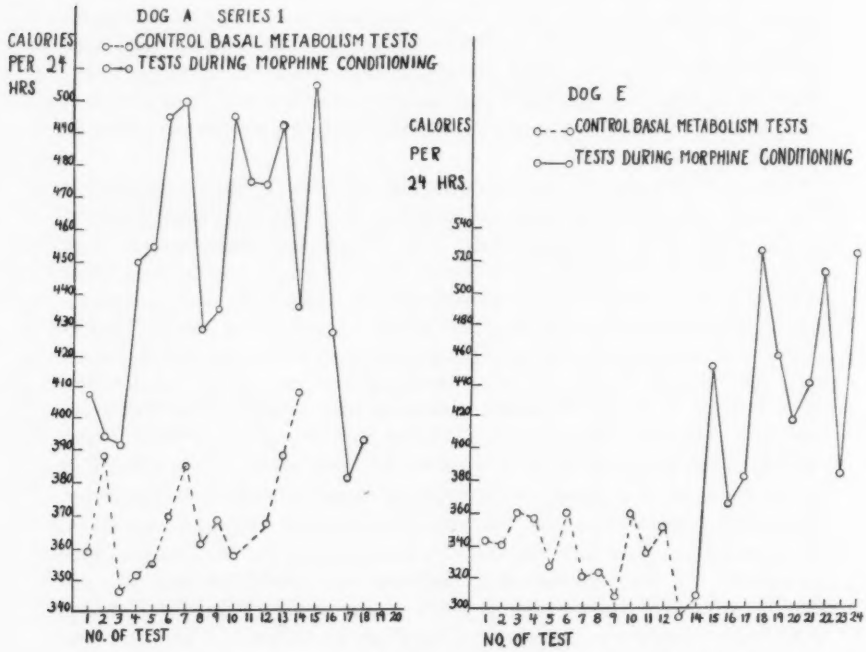
per cent; E, 33.64 per cent. (See table 1.) These figures do not include tests in which the dogs started to rise or made similar marked movements, since it has been shown (Benedict, 1924) that muscular exertion may increase the metabolic rate over the basal level. Tests were retained, however, in which slight movements were visible, since Benedict reports that moving the hand to the face once or twice a minute does not modify the basal metabolism, and that raising the hand to the face every four seconds gives an increase of plus 6 per cent to plus 11 per cent over the average basal metabolic rate. The slight movements were not observed in the dogs as often as every four seconds, and if they had occurred, would not explain the large increases (up to 35 per cent over the average basal metabolic rate) often found in the morphine tests. Also the fact that there were high increases over the basal metabolic rate when no movement was observed as well as when slight movements were observed, indicated that these slight movements were not responsible for the large increases found in the morphine test periods.

Since protocols showed that conditioning, as judged by salivation, did not begin until after three injections of morphine, the first three tests followed by injections were also discarded. The remaining morphine tests were characterized by variability from test to test (see graphs) sometimes being 25 per cent to 35 per cent above the average basal metabolic rate, and sometimes falling within the control period range. Therefore it seemed important to determine whether or not there was any significant difference between each control period and the following morphine test period. A method employed by R. A. Fisher (1928) in dealing with a small number of cases was used. Fisher's formula applied to the data of a given control period and morphine test period, gives a value "P" (see table). This value P is the probability of drawing two compared samples, with their respective means and variabilities, from the same distribution. When P = 0.01−, the probability is less than one out of a hundred that the two groups(a control period and the corresponding morphine test period, for example) are random samples of a single homogeneous group. Such a value for P means that the two samples in question are significantly different. The first test period was in all cases significantly different from the control period as shown by P = 0.01− in each case.

B. *Spontaneous changes in the metabolism accompanying the conditioned response to morphine.* In series 2 where the morphine injections were repeated in dogs A and B, there is evidence for a spontaneous decrease in the metabolism response on repeated conditioning of the animal. In dog B, in both series 2 and 3, the average of the test period is actually lower than the average for the corresponding control period. The values for P indicate that there is little or no difference between the test periods and control periods. In dog A there is less striking a decrease in the response, the

difference between test and control period averages being 87.32 cals. in series 1, and 62.37 cals. and 68.08 cals. respectively in series 2 and 3.

C. *Artefacts due to swallowing air.* The data show that oxygen consumption values were not enlarged by swallowing air with saliva. In dog A, the differences between control period and test periods are practically the same in series 2 (just before removal of the salivary glands) and in series 3 (after removal of the salivary glands). In dog B the average of the test period in series 2 is lower than that of the control period of the same series, even though the salivary glands were present and some salivation was



noted. In this dog, after removal of the salivary glands, in series 3, there is a further slight reduction of the test period average below the control period average, but this would seem insignificant as compared to the spontaneous drop occurring between series 1 and 2 in this same dog.

D. *Adrenal gland denervation.* In view of the spontaneous decrease in the increments over the basal metabolic rate occurring with repeated conditioning of the animals, denervation of the adrenal glands was carried out in two dogs which had never received morphine injections. A comparison was then made of their first metabolism response on morphine conditioning

and the first metabolism responses of the three dogs with adrenal innervation intact. The differences between control period and test period averages in series 1, for the five dogs are: normal dogs, 23.7 per cent, 28.0 per cent and 13 per cent; operated dogs, 22.2 per cent and 33.64 per cent. One of the operated dogs, it is seen, is near the highest normal dogs, and the other operated dog shows the greatest percentage increase of the five dogs studied. It might be expected that if adrenal denervation ruled out a hormone of importance in producing the increased metabolisms of the test periods, dogs D and E (operated) should show considerably less rise over the basal metabolic rate than dog B (unoperated).

A comparison of the morphine test periods in dog A before and after his adrenal glands were denervated, is also relevant. In series 3, before denervation of the adrenals, there is a difference between control and test period of 68.08 cals., while in series 4, after denervation, the difference is 47.77 cals. In view of the spontaneous fall in the response between one series and the next already observed in this dog, and in dog B, the most significant values seem to be those given above, where normal and operated dogs are compared on first being conditioned to morphine.

Two dogs with adrenals denervated were killed five weeks after the last stage of the operation, removal of 1 to 2 inches of thoracic sympathetic chain above the diaphragm. Gross examination showed connective tissue where the chain had been. Histological examination showed no evidence of nerve fibers regenerating in the area. The denervated experimental dogs were showing a definite increase in the metabolism over the basal level an equal time after operation.

Discussion. The results presented indicate that test conditions associated with the giving of morphine induce a state of excitement which is accompanied by marked increases in metabolism over the basal metabolic rate. There are certain practical bearings of this experiment, for it is possible that in taking ordinary basal metabolism determinations on dogs, incidental stimuli occurring during or after the test may lead to disturbances of the basal metabolic rate, and account for some of the variability reported in basal metabolism studies.

Relation of the adrenals to basal metabolic rate. It has been reported by various workers that basal metabolism is lowered by procedures said to remove adrenal secretion. Aub, Forman and Bright (1922a) studied one cat with the right adrenal removed and the left splanchnic nerves cut. They report a fall of 12 per cent in the metabolism below the basal level 37 days after the operation. They conclude that removal of one adrenal and denervation of the remaining adrenal is followed by a slow fall of the metabolic rate. The same workers (1922b) removed both adrenal glands and determined metabolism under urethane anesthesia, and found a "prompt and progressive fall of metabolism which averages 12 per cent."

Since "adrenalin injected intravenously at a physiological rate causes a distinct rise in the metabolism" the conclusion is suggested that the fall in metabolism after adrenalectomy was due to lack of secretion from the adrenal medulla. McIver and Bright (1924) found an average drop of 7 per cent in the metabolism of animals under urethane anesthesia following removal of the adrenals.

Three dogs studied in this paper had the remaining adrenal medulla presumably removed from nervous influences. Two of the three dogs had average basal metabolism values in calories per square meter of surface area, which were in the higher part of the normal range. The third dog had a basal metabolism lower than the other dogs tested, yet well within the normal range. These results do not indicate a lowering of the basal metabolism following the denervation technique employed.

Extra adrenalin and metabolism. It has been reported by Boothby and Sandiford (1923) that adrenalin injected intravenously into dogs increases the metabolism. Adrenalin chloride injected into normal dogs at rates claimed by Cannon and Rapport (1921) to be within physiological limits gave increases of 25 per cent to 35 per cent over the average basal metabolic rate. Hunt and Bright (1926) gave intravenous injections of adrenalin at a rate slightly above the maximum physiological increase as determined by Cannon and Rapport. The peaks of the rises of metabolism averaged 19 per cent over the control level. In a paper by Cannon, Querido, Britton and Bright (1926), it is stated, that "since injected adrenalin increases metabolism, and adrenalectomy decreases it, the inferences seem justified that absence of the medulla and its secretion, rather than absence of the cortex, is responsible for slower metabolic rate after removal of the adrenals; and conversely that secreted adrenin helps to maintain the normal metabolic rate and by a larger output may increase that rate."

According to Cannon and Britton (1926) some of the circumstances which lead to the secretion of adrenalin, as indicated by a faster rate of the denervated heart, are slight muscular contractions, strenuous exertion, minor excitement and strong "emotion," the two latter produced in a cat by the presence of a barking dog. Since in these experiments the liver and thyroid were also denervated, the question arises as to whether or not adrenal secretion has an important function in normal animals.

Some evidence on this point has been presented in a study of animals working on a treadmill (Campos, Cannon, Lundin and Walker, 1929). It was found that if one adrenal was removed and the other denervated, the capacity for work was not decreased as compared with normal dogs. The comment is made that "it was not at all expected that after removal of one adrenal and demedullation of the remaining gland the energy output would be as great as under standard conditions." The authors give the explanation that possibly "the normal animal does not use that (adrenal)

apparatus to any noteworthy degree in routine running, but only in unusual emergencies." However since the dogs were exercised until they refused to run and often were unable to stand, they would seem to be in a situation which would be generally classed as an emergency.

The results reported above also bear on the question of adrenal function in normal animals. Those dogs with one adrenal removed and the other denervated, showed as great an increase over the basal metabolic rate during the conditioned state as did the unoperated dogs. According to the reports of Cannon and Britton, cited above, adrenalin might be expected to be secreted during the conditioned response induced by morphine, but if such a secretion occurred, it was not necessary for the increases over the basal metabolism which were found. It must be assumed that if in the normal dogs adrenalin was secreted in excess of basal amounts during the conditioned excitement period, and caused some of the increased metabolism observed, then in the operated dogs if no excess adrenalin was secreted, its stimulating action on metabolism was completely taken over by some other agency.

CONCLUSIONS

1. Increases in metabolism over the basal metabolic rate accompany the conditioned state induced in dogs by morphine. It is shown that these increases are greater than chance variations from the basal metabolic rate.

2. The degree of increase over the basal metabolic rate during the conditioning period varies from dog to dog.

3. After allowing time for disappearance of the conditioned state a second or third induced conditioned state is accompanied by less increase over the basal metabolic rate.

4. After removal in dogs of one adrenal gland and denervation of the other no decrease in basal metabolic rate occurred during the control periods. During the conditioned state increases over the basal metabolic rate occurred as in normal dogs. Discharge of adrenalin seems not to be a necessary factor in this increase.

I wish to express my appreciation to Dr. A. J. Carlson, under whom this work was done, for his advice and criticism.

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THE INFLUENCE OF ATROPIN AND PILOCARPIN ON THIRST (VOLUNTARY INGESTION OF WATER)

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The importance of local dryness of the mouth and pharynx as the exciting cause of the sensation of thirst, apart from any general bodily dehydration, has long been a subject of controversy. Cannon (1) states that when he dried up his buccal secretions by means of atropin he definitely experienced thirst. He did not drink, and the thirst disappeared when the atropin effect wore off. L. Mueller (2) reports that his experimental subjects were able to differentiate clearly between thirst and the dryness of the mouth induced by atropin. Siebeck (3) is inclined to agree with E. Meyer (4) that one ought definitely to call the sensation which arises after the administration of atropin, thirst, but admits that in the final analysis it is a question of the definition of the concept "thirst."

The converse of the atropin experiments, namely, attempts to alleviate thirst artificially by inducing a free flow of saliva with pilocarpin, has been reported a number of times. Pack (5) withheld water and food from rabbits for seven days. At the end of that time some of the rabbits were injected with 0.5 cc. of a 1 per cent solution of pilocarpin hydrochloride subcutaneously. When salivation had become profuse, water was offered for one hour. The controls which were given no pilocarpin drank 62 to 137 cc. of water in one hour, while the pilocarpin injected animals drank only 0–25 cc. in the same period of time. Schneider (6), Wiethe (7), and Wisotzki and Eymueller (8) report relief in some cases of post-operative thirst by the use of the sialogogues Cesol and Neucesol. On the other hand, Weir, Larson and Rowntree (9) were unable to relieve thirst in four patients with diabetes insipidus by injections of pilocarpin.

In a recent paper (10) I reported that complete extirpation of the salivary glands in dogs did not lead to an increased fluid intake, and concluded that the salivary glands cannot play as important a rôle in the thirst mechanism as Cannon ascribes to them. I therefore thought it very unlikely that atropin could cause thirst or pilocarpin relieve it simply by changes in the degree of moistness of the mouth. However, the possibility remained that these drugs might affect water ingestion by some other means, as for example, by their effect upon water distribution in the body.

The following experiments were performed in the hope of showing definitely 1, whether atropin and pilocarpin have any influence upon water ingestion, and 2, whether any such effects are related to the presence or absence of a free flow of saliva.

METHODS. In all six dogs were used, three normal animals and three in which the salivary glands had been extirpated. They were placed in metabolism cages in a room where they were not subjected to wide fluctuations of temperature and were fed equal parts of meat and bread. The quantity of food eaten was kept as nearly constant as possible. Water intake and urine were measured. The following experiments were performed on these animals:

A. *Experiments with atropin.* 1. The effect on water intake of the repeated administration of atropin over a period of three days. Two normal and two operated dogs were used. Control observations were made for eight days preceding and nine days following the administration of the drug. On the three days of the experiment a total of five doses of 0.14 mgm. per kgm. of atropin was given subcutaneously. One dose was given at 2:30 p.m. on the first day, and on the second and third days, injections were made at 10:00 a.m. and 5:00 p.m. The daily water intake and the output in urine and feces were measured.

2. The effect upon the two-hourly water ingestion of atropin in single doses of 2 to 20 mgm. Three normal and two operated dogs were used. On four to five control days the water drunk was measured every two hours from 8:30 a.m. until 6:30 p.m. On the days of the experiment the atropin was given subcutaneously at 10:30 a.m., and the water intake was measured every two hours until 6:30 p.m. The dogs were fed daily at 4:30 p.m.

B. *Experiments with pilocarpin.* 1. The influence of pilocarpin upon water ingestion after a two day period of water deprivation. Two normal and three operated dogs were used. The water intake was measured for eight days. Then water was withheld for two days, food being given as usual. On the morning of the third day 0.6 to 0.7 mgm. per kgm. of pilocarpin was given subcutaneously, and water was offered. The fluid intake was recorded the second and fourth hours after the injection, and then daily until it returned to the normal level.

2. The effect of pilocarpin upon water ingestion after a two day period of fasting and water deprivation. This experiment was performed in the same way as the preceding one except for the fact that food was withheld as well as water. Two normal and two operated dogs were used. The experiment was performed twice. Each time only one normal and one operated dog were injected with pilocarpin, while the others acted as controls.

RESULTS. A. *Experiments with atropin.* 1. The results of the repeated administration of small doses of atropin over a period of three days are

presented in table 1. In only one instance (dog 10) was the average water intake greater during the experimental than during the control period. The average increase of 15 cc. appears to be insignificant because the daily intake during the experimental period remained within the limits of the daily fluctuation observed during the control period.

2. When single large doses of atropin were administered in the morning to dogs with and without salivary glands the effect upon the two-hourly intake of water throughout the day shows no change indicative of the production of thirst as a result of the injection. Doses of 5 mgm. were given on two days, a dose of 10 mgm. on one day and finally 20 mgm. on three days. Between experimental days, time was given for recovery from the effect of the drug. Since the results obtained with the smaller

TABLE 1

The influence upon water intake of repeated small doses of atropin over a period of 3 days

	NORMAL DOGS						DOGS WITHOUT SALIVARY GLANDS					
	Dog 8, weight 13.8 kgm.			Dog 10, weight 10.6 kgm.			Dog 3, weight 10.9 kgm.			Dog 4, weight 11.4 kgm.		
	Food	Water drunk	Water in urine and feces	Food	Water drunk	Water in urine and feces	Food	Water drunk	Water in urine and feces	Food	Water drunk	Water in urine and feces
	gram	*cc.*	*cc.*	*gram*	*cc.*	*cc.*	*gram*	*cc.*	*cc.*	*gram*	*cc.*	*cc.*
Control period, average 8 days	400	155	202	365	230	148	285	157	147	400	200	169
Experimental period, average 3 days	400	153	226	390	245	143	300	140	90	400	173	307
Control period, average 9 days	400	200	231	370	225	165	300	170	113	400	166	234

dosages are in every way comparable with the effects of the 20 mgm. dose, the observations of the latter alone are given in detail in table 2.

It will be seen that in the case of a single dog without salivary glands (dog 3) the average ingestion of water was considerably greater during the two hours following the injection than in the same two hours during the control period. An increase at the same hour was observed in this same dog in one instance after a 5 mgm. dose of atropine. In all other cases the fluid intake during this period was lower than usual. Minor fluctuations up or down were noted during the next four hours up to the time of feeding. The total intake for 24 hours was higher after atropin in three dogs (two with and one without salivary glands) and lower in two dogs (one with and one without salivary glands). The daily fluctuations were within normal limits. No difference in the behavior of the two groups of

TABLE 2

The effect of single large doses of atropin upon the two-hourly fluid intake

	NORMAL DOGS						DOGS WITHOUT SALIVARY GLANDS			
	Dog 8, weight 13.8 kgm.		Dog 10, weight 10.6 kgm.		Dog 11, weight 11.0 kgm.		Dog 3, weight 10.9 kgm.		Dog 4, weight 11.4 kgm.	
	Control period	Experimental period	Control period	Experimental period	Control period	Experimental period	Control period	Experimental period	Control period	Experimental period
Atropin at 10:30 a.m.	0 mgm.	20 mgm.	0 mgm.	20 mgm.	0 mgm.	15–20 mgm.	0 mgm.	20 mgm.	0 mgm.	20 mgm.
Water intake, average of	4 days	3 days	5 days	3 days	5 days	3 days	5 days	3 days	5 days	3 days
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
8:30–10:30 a.m.	0	8	17	12	43	105	17	15	9	27
10:30–12:30 p.m.	0	0	11	7	17	3	24	53	20	0
12:30– 2:30 p.m.	4	0	0	0	10	17	19	0	13	0
2:30– 4:30 p.m.	19	3	2	0	15	3	0	7	13	26
4:30– 6:30 p.m. (after food)	220	10	29	32	87	3	74	3	36	19
Total intake 24 hours	345	370	171	258	354	185	220	175	177	192
Average food intake, grams	400	400	360	330	320	300	370	300	390	400

animals was observed which could be interpreted as meaning that atropin produced an increased desire for water by inhibiting salivary secretion and increasing the local dryness of the mouth.

B. *Experiments with pilocarpin.* 1. The results of administering a fairly large dose of pilocarpin to normal and operated dogs after having deprived them of water for a period of two days are recorded in table 3. Far from being relieved of thirst by the free flow of saliva which pilocarpin induced, the normal dogs drank more water during the first four hours after injection and also during the 24 hour period. The emetic action of

TABLE 3

The influence of pilocarpin upon water intake after a two day period of water deprivation

	OPERATED			NORMAL	
	Dog 3, weight 10.9 kgm.	Dog 4, weight 11.4 kgm.	Dog 7, weight 7.3 kgm.	Dog 8, weight 13.8 kgm.	Dog 10, weight 10.6 kgm.
Pilocarpin	7 mgm.	7 mgm.	5 mgm.	10 mgm.	7 mgm.
	cc.	cc.	cc.	cc.	cc.
Control period (average of 8 days):					
Water drunk	156	174	100	190	215
Water in urine and feces	120	187		195	145
Water only withdrawn for 2 days; pilocarpin given on third day:					
Water taken in					
First and second hour	140	165	180	295	300
Third and fourth hour	105	90	0	205	155
Twenty-four hours	385	425	305	830	755
Water in urine, feces, vomitus	100	150		465	300
Vomiting	None	Slight	Slight	Moderate	Marked
Salivation	0	0	0	++	+++

the pilocarpin appeared to be more marked in the case of the normal animals. The amount of fluid ingested minus vomitus and urine is of the same order of magnitude in the two groups of animals.

2. In this experiment only two of the four animals used were injected at the same time. The other two acted as controls. See table 4. In the first experiment one notes that the dogs, normal and operated alike, which received pilocarpin, drank more than the other two dogs during the height of the pilocarpin effect. In the second experiment, dog 3, an operated dog which received pilocarpin, drank less during the first hour than dog 4

(also operated), which had not received pilocarpin. However, in the case of the normal dogs, no. 11, which had received pilocarpin, drank more than no. 10, which had not.

Comparing the reactions of the individual dogs in the two experiments we see that one of the dogs without salivary glands (no. 3) drank less when pilocarpin was injected after a period of thirst than when the drug was not

TABLE 4

The influence of pilocarpin upon water intake after withdrawal of food and water for a period of two days

	OPERATED		NORMAL		OPERATED		NORMAL	
	Dog 3	Dog 4	Dog 10	Dog 11	Dog 3	Dog 4	Dog 10	Dog 11
Pilocarpin dosage	0 mgm.	7 mgm.	7 mgm.	0 mgm.	7 mgm.	0 mgm.	0 mgm.	8 mgm.
	Average of 10 days				Average of 3 days			
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
Control period:								
Water drunk	255	253	241	239	213	195	195	302
Water in urine and feces	125	368	182	198	171	310	150	198
Food and water withdrawn for 2 days; pilocarpin, food and water given on third day								
Water drunk:								
First hour	260	360	330	240	105	130	60	310
Second hour	0	0	0	0	5	50	0	250
Third and fourth hour	0	0	0	0	60	35	30	80
In 24 hours	690	695	790	535	540	395	370	1,020
Urine	35	230	100	200	150	300	275	
Vomiting	0	Slight	380	0	Slight	0	0	470
Salivation	0	0	+++	0	0	0	0	+++

given. The other dogs all drank more. These experiments show that in spite of the variations in the reaction of individual dogs to injections of pilocarpin, no evidence whatsoever has been obtained to show that pilocarpin relieves thirst by promoting a free flow of saliva. The only dog which drank a smaller quantity of water after an injection of pilocarpin was one which had been deprived of salivary glands.

CONCLUSIONS

1. Repeated injections of small doses of atropin over a period of three days did not significantly alter the water intake of dogs with or without salivary glands.

2. Large single doses of atropin (up to 20 mgm.) did not significantly alter the two-hourly fluid intake of these dogs.

3. Injections of pilocarpin, after a two day thirst period, did not diminish the water intake of normal dogs as compared with that of dogs which had been deprived of their salivary glands.

4. It, therefore, appears likely that whatever changes in water ingestion may occur after injections of atropin or pilocarpin are caused by some other factor than drying or moistening of the mucous membranes of the mouth and pharynx by suppression of or increase of the salivary secretion.

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RESTORATION OF THE PANCREATIC SECRETION BY PEPTONE AND HISTAMINE

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Rasenkow (1929) has shown that in a dog, when the response of the pancreatic gland to repeated injections of HCl into the duodenum has become exhausted, the introduction of certain cleavage products of proteins, such as albumoses and peptones, has the effect of restoring the pancreatic reaction to HCl. These products introduced into the duodenum alone did not activate a pancreatic secretion. Further, amino acids did not exhibit this restorative action. The loss of the secretory effect of HCl solutions after repeated injections cannot be attributed to the exhaustion of secretin in the mucous membrane. Rasenkow found that, when the mucous membrane of a dog which failed to secrete was extracted with 0.5 per cent HCl and injected into a second dog, it produced a copious flow of juice similar to that activated by a normal duodenal extract.

Koschtojanz (1929) continued this investigation and found that acid digests of meat and milk in gastric juice had an effect similar to that of peptones and albumoses in restoring the action of acid. A similar digest of bread, however, did not have this property.

Since the usual commercial preparations of peptone contain considerable quantities of histamine (Feldberg and Schilf, 1930), and as this amine is probably present in digests of proteins, it was considered that histamine might possibly play a part in these experiments in restoring pancreatic secretion. Furthermore, histamine is present normally in the gastrointestinal tract (Best and McHenry, 1930), and it has been demonstrated that it exerts a secretory action on the pancreatic gland (Dale and Laidlaw, 1910; Popielski, 1920; and others).

The present investigation was undertaken to determine whether histamine plays any part in the restoration of the pancreatic secretion when it has been exhausted by repeated injection of HCl into the duodenum.

METHODS. Dogs and cats were used, the animals being anesthetized first with ether and then by injection into the saphenous vein of a mixture of chloralose and urethane (0.05 gm. chloralose and 0.5 gm. urethane per kilo body weight). The carotid blood pressure was registered in the usual

way. The pancreatic duct was cannulated and connected to a Gibbs drop recorder. In all the experiments the bile duct and pylorus were ligated.

In the first experiments a cannula for the introduction of 0.2 per cent hydrochloric acid was inserted into the first part of the duodenum only, and all the acid injected remained in the intestines. This procedure caused an undesirable distention of the small intestine, and in the later experiments a second, outlet cannula was inserted in the upper ileum about 18 to 24 inches from the pylorus.

EXPERIMENTAL RESULTS. *Effect of diet.* Previous feeding of the animals was found to play a very important part in these experiments. If the animals had been fed the day before the experiment, and especially if there were food particles in the duodenum, it was practically impossible to exhaust the response of the pancreas to repeated injection of acid (0.2 per cent HCl). Conversely, if the animals had been given water only for more than 24 hours, there was practically no response whatever to HCl. Our standard procedure was to use animals which had been previously fed on a diet of meat and milk and which had received no food but had been given water ad lib. for 18 to 24 hours. In the case of cats 10 cc., and in the case of dogs 20 to 30 cc. of 0.2 per cent hydrochloric acid were injected repeatedly into the duodenum.

Action of histamine on the pancreatic secretion. When the pancreatic gland failed to respond to 0.2 per cent HCl solution, histamine in aqueous or acid solution (0.2 per cent HCl) was injected into the duodenum two or three times at 15 to 20 minute intervals. Usually 10 mgm. of histamine in 10 cc. of fluid were injected in the cats, and 20 to 30 mgm. of histamine in 20 to 30 cc. of fluid in the dogs. In most of the experiments histamine was injected in acid solution, so that there would be a constant stimulus present in the duodenum. We observed that histamine injected under these conditions had the effect of restoring the response to HCl in 15 to 60 minutes. The positive action of histamine was slower in the experiments where there was no drainage of the small intestine, owing probably to the longer time required for the histamine solution to displace the acid in the filled loops of the intestine and become distributed over the duodenum and jejunum. Also, in these experiments the restored response to HCl stimulation persisted for a much longer period, viz., 2 to 4 hours. In some experiments, when the duodenal-jejunal loop was drained, the restorative action of histamine was almost instantaneous, i.e., the first injection of histamine in HCl gave a positive effect in augmenting the secretion. In these experiments the effect of histamine was lost after two or three subsequent injections of HCl, as the histamine was washed out of the loop. However, when the secretion from HCl stopped and histamine was again introduced, the secretion again appeared. This cycle was observed as many as three or four times in the same experiment before the mucous

membrane became permanently damaged. These results are illustrated in figure 1. Rasenkow's observation concerning the restoration of the pancreatic secretion by 2 per cent peptone solution (Witte's peptone) was also confirmed by us.

In the few experiments where histamine had no positive effect, the addition of peptone produced no result. In these cases it was usually found that the intestinal mucous membrane was seriously damaged so that extensive desquamation had taken place. It was generally noted that the earlier the preliminary exhaustion to HCl occurred, the quicker and more pronounced was the restorative response to histamine.

Absorption of histamine from the small intestine. During the course of these experiments it was interesting to note evidence of the absorption of histamine from the small intestine, as this is a controversial point. The

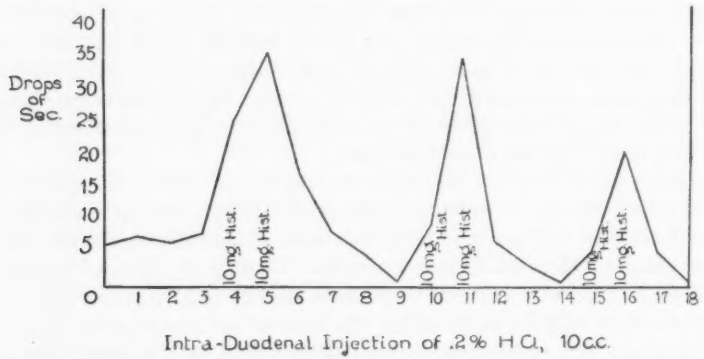

Fig. 1. Restoration of pancreatic secretion in a cat by the addition of 10 mgm. histamine to injections of 0.2 per cent HCl.

blood pressure was used as an index of absorption. Meakins and Harington (1925) found that in cats anesthetized with paraldehyde there was a sudden marked fall in blood pressure on introducing histamine into the small gut. Wangensteen and Loucks (1928) were unable to verify this result in dogs. Mellanby (1915) in an exhaustive study of the problem in cats noted that there was a rapid disappearance of histamine from the small gut and a gradual but profound fall in blood pressure. However, Mammoser (1929) and MacKay (1930) did not observe any change in the blood pressure after the introduction of large amounts of histamine (up to 100 mgm.) in aqueous solution into the small intestine of dogs and cats. On the other hand, Mammoser noted a prompt and prolonged lowering of the blood pressure if histamine (5 mgm. per kgm.) was introduced when the gut had previously been treated with chemical agents such as chloroform, ethyl alcohol and 0.4 per cent HCl.

In a series of experiments in which 10 mgm. of histamine in 0.2 per cent HCl were introduced into the upper part of the small intestine in cats, there was a more or less prompt fall in blood pressure lasting from $\frac{1}{2}$ to $\frac{3}{4}$ of an hour. Thus it would appear that histamine is absorbed from the small gut in acid solution but not in aqueous solution (fig. 2).

Discussion. Since histamine is usually contained in commercial peptone and in the digestive products of meat and milk, it may be considered as one of the factors in restoring the pancreatic secretion. To determine the respective parts played by peptone and histamine in this restorative process, further research is necessary. The exact mechanism involved is

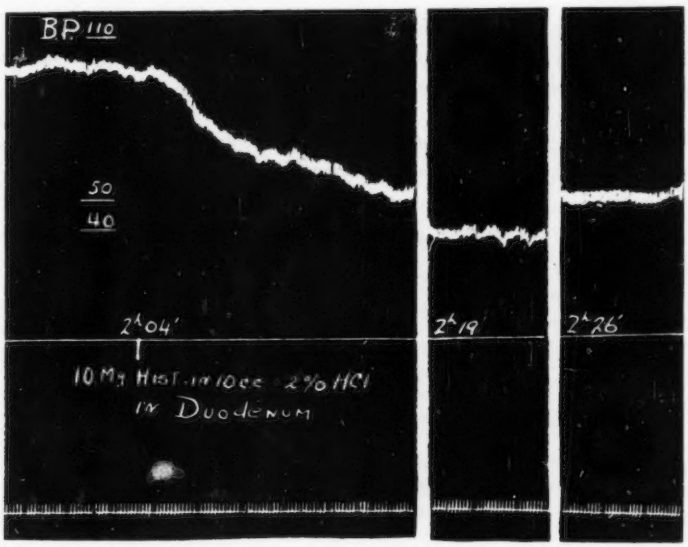

Fig. 2. Fall of blood pressure in a cat after the injection of 10 mgm. histamine in 10 cc. 0.2 per cent HCl into the duodenum.

not clear, but it would seem that histamine not only acts as a secretory stimulus but has also some effect on the secretin-containing mucous membrane of the duodenum and jejunum whereby the secretory reaction to HCl is restored. It is unlikely that histamine absorbed from the gut in acid solution exerts a direct action only on the pancreatic gland, as in these experiments the return of the secretion was very copious and lasted for long periods. On the other hand, intravenous injection of 3 to 5 mgm. of histamine in a cat gives only a few drops of secretion. Further, the action depends on the integrity of the intestinal mucosa, no effect being noted when the latter is damaged. The fact that histamine is absorbed

from the small gut in acid but not in aqueous solution under the experimental conditions described above, emphasizes the necessity of investigation of the part played by histamine in the pancreatic secretion under perfectly normal conditions.

SUMMARY

1. When the pancreatic response to repeated injections of 0.2 per cent HCl has become exhausted, it may be restored by injecting into the duodenum 0.2 per cent HCl to which 10 to 30 mgm. of histamine have been added.

2. The previous feeding of the animals is an important factor in these experiments.

3. Histamine is absorbed from the small intestine when injected in 0.2 per cent HCl, or when injected in aqueous solution if the gut has been subjected to previous injections of HCl.

This investigation was carried out under the direction of Dr. B. P. Babkin, to whom the writers are indebted for much valuable criticism and advice.

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STUDIES IN DECEREBRATION

VI. The Effect of Deafferentation upon Decerebrate Rigidity

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Proprioceptive reflexes from the muscles play a rôle in the production of the increased extensor tone which is characteristic of decerebrate rigidity. Just what the proportion of this rôle is in relation to other factors which influence the rigidity is as yet undetermined.

Sherrington (1) has stated that "decerebrate rigidity is simply reflex standing." He found that section of the posterior spinal roots in a decerebrate cat was followed immediately by disappearance of the rigidity. In elaborating this conception Fulton (2) said, "Now the postural response which gives rise to decerebrate rigidity *is* the stretch reflex. In the elicitation of the stretch reflex, therefore, we have 'tonus in its making.' "

Other investigators, Liljestrand and Magnus (3), Ranson (4), Spiegel (5) and Pollock and Davis (6) have found that posterior root section does not prevent the occurrence of rigidity after decerebration. However, in all of these experiments, including our own, although the extremity studied was deafferented completely impulses which originated from other extremities and the trunk could modify the reflex activities of the deafferented extremity. An experiment was planned to preclude such a possibility.

Experiment. In a group of four cats, the first 23, 20, 18 and 21 posterior spinal roots were divided intradurally upon both sides. This procedure required as many as three successive operations upon the same animal. As a result, in each animal both forelegs were deafferented completely. Three weeks after the last operation the animals were decerebrated by the anemic method. This was followed by transection of the spinal cord at a level one segment above the last pair of sectioned roots.

Results. In contrast to the ordinary acute spinal animal, no evidence of spinal shock was observed. Stepping movements occurred immediately after the operation and contralateral extensor responses were elicited promptly. It is obvious that in these animals reflex activities could be elicited only by stimulation of the labyrinths and the head.

After decerebration, the animals developed marked extensor rigidity

in all extremities. This persisted in the forelegs after the spinal cord was sectioned. As long as the animal's head remained in a position with the occiput down the rigidity persisted. It was necessary to exert a pressure of 800 grams to produce flexion of the extended forelegs. Turning the head to one side was followed by diminution of extensor tone in the opposite foreleg (fig. 1).

Discussion. In conformity with our former experiments (6), we found that tonic reflexes and those elicited by nociceptive stimuli to parts of the body with uninterrupted afferent pathways (the head) were facilitated. The tonic labyrinthine reflexes were brusque and forceful. Grasping the head increased the extensor rigidity. The pinna, head-shaking, sneezing and respiratory reflexes were very brisk.

Measured by the standard of persistence of extensor rigidity, the tonic muscles were indefatigable. When subjected to repeated rapid flexions,

Fig. 1. Photograph of a decerebrate cat with bilateral section of 23 posterior spinal roots and transection of the spinal cord.

each succeeding flexion required less force, until practically no tone remained in the extensor muscles. This might be interpreted as fatigue or to a quality of extensibility in which the muscle is "pulled out like a gum," as we described in a former communication (7). The extensor muscles of otherwise normal decerebrate animals show this characteristic to a lesser degree.

We (6) have shown that the labyrinths exert a strong influence upon the extensor reflex of the neck, which tends to produce a fixed position of the head in extension and a subsequent marked extensor rigidity in the forelegs. On the other hand, the extensor tone in the hind legs seems far less dependent upon the labyrinthine reflexes and somewhat more upon the neck reflexes. Flexion of the head produces strong extension of the hind legs. The neck reflexes directly contribute largely to the rigidity of the forelegs. This is flexor in type when the head is flexed and extensor in character

when it is extended. When the labyrinthine and neck reflexes were destroyed and the remaining rigidity was dependent largely upon proprioceptive reflexes from the muscles, more extensor tone was found in the hindlegs than in the forelegs where only a slight amount of rigidity was present. Good extensor rigidity could be produced, however, by attempts to flex the extremities. The amount of rigidity in such a preparation is far less than in one in which the neck or labyrinthine or both of these reflex activities are conserved.

The present experiment demonstrates that in so far as the forelegs are concerned strong extensor rigidity in a decerebrate animal is produced directly by labyrinthine reflexes without the existence of any tonus produced by a stretch reflex. Decerebrate rigidity is due, therefore, to a summation of reflex activities, of which the stretch reflex represents but a small part.

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THE METABOLISM OF SKELETAL MUSCLE UNDERGOING ATROPHY OF DENERVATION[1]

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This report is concerned with the results of experiments carried out to determine whether skeletal muscle, which had been deprived of its nerve supply and allowed to undergo atrophy, exhibited a metabolism similar to that of normal intact muscle. A comparison was made of the metabolism of normal and denervated limbs of dogs under the conditions of rest, exercise, glucose administration and after the subcutaneous injection of adrenalin. This was done by comparing the composition of blood entering and leaving a denervated limb with that from the opposite normal limb. Blood was analyzed for glucose and lactic acid. In addition the oxygen and carbon dioxide contents of the blood were determined in order that the respiratory quotients might be calculated.

EXPERIMENTAL METHODS. Large adult dogs were operated upon under amytal or ether anesthesia. An incision was made along a line from the first to the third trochanter of the femur and a section of the sciatic nerve, about 4 cm. in length, was removed. A similar section of the femoral nerve was made at the ligament. Studies were made upon these animals at periods varying from one-half hour to 14 weeks after denervation. The femoral artery and veins were exposed under amytal or local procaine anesthesia. Simultaneous drawings of the venous bloods were made under paraffin oil and discharged into tubes containing oxalate and fluoride. The arterial blood was drawn alternately before and after the venous drawings. It was found that one satisfactory drawing could be made under local anesthesia. Because of the presence of scar tissue, amytal anesthesia (60 mgm. per kilo, intraperitoneally) was found to be more satisfactory for subsequent vein exposures. In order to determine the respiratory quotient of the entire animal, the expired air was collected for a period of 10 minutes in a Tissot spirometer and analyzed by the Haldane apparatus. Blood sugar was determined by the method of Shaffer and Hartman (1920) on filtrates prepared according to Somogyi (1930). The lactic acid was determined, in duplicate, according to the method of Friedmann, Cotonia

[1] Aided by a grant from the Laura Spelman Memorial Fund.

and Shaffer (1927). All blood samples were analyzed for their CO_2 and O_2 content by the method of Van Slyke and Neill (1924) and checks were obtained to 0.2 volume per cent. Blood was drawn before and one hour after the subcutaneous injection of $\frac{1}{5}$ mgm. of adrenalin per kilo of body weight. The exercise was induced by the application of induction shocks through needle electrodes to the muscle groups at the rate of two per second. The feet were weighted with a light load to restrict the extent of movement. The exercise was continued for a period of 20 minutes and was in progress at the time blood was drawn. Glucose was given by stomach tube in amounts of 2 to 3 grams per kilogram of body weight $1\frac{1}{2}$ hours to 2 hours before blood was drawn.

RESULTS. *Lactic acid.* The values for the lactic acid content of arterial and venous blood from the normal and denervated limbs are given

TABLE 1

Average values for the sugar and lactic acid content of arterial and venous blood from normal and denervated limbs

CONDITION	LACTIC ACID			SUGAR	
	Arterial	Control	Denervated	Control limb, A-V difference	Denervated limb, A-V difference
	mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
Resting	16.6	17.1	17.1	−3.8	−3.1
Exercise	17.5	15.1	17.1	1.4	−0.4
1 hour after adrenalin	37.1	37.6	37.2	−3.0	−2.2
After glucose				−3.6	−4.8
Average of all determinations				−2.9	−2.8

in table 1. The average values for the resting condition show a slightly greater lactic acid content in the venous blood. In six out of ten experiments both normal and denervated limbs were adding lactic acid to the blood stream. One hour after the subcutaneous injection of $\frac{1}{5}$ mgm. of adrenalin per kilogram of body weight the values for the lactic acid content of the blood had more than doubled. The lactic acid content of the venous blood from the control limbs was greater than that of the arterial in 3 out of 5 experiments. The lactic acid content of the venous blood from the denervated limbs was higher than that of arterial in 2 out of 5 instances. In the experiments in which the muscle groups were stimulated with two induction shocks per second for a period of approximately 20 minutes, the venous blood did not show an increase in lactic acid. In fact, in 4 out of 5 experiments the concentration of lactic acid in the venous blood was lower than in the arterial. The oxygen differences between arterial and venous

bloods were more than double that noted in the resting condition, yet no lactic acid was added to the blood stream by the muscles of either the normal or denervated limbs. When considered as a whole, the experiments show that the lactic acid metabolism of skeletal muscle undergoing atrophy of denervation is essentially the same as that of normal muscle as far as can be determined from analysis of blood entering and leaving the limbs.

Blood sugar. The average value (table 1) for the sugar content of venous blood was slightly lower than that of arterial in all conditions except that of exercise. The differences were of the same magnitude in the blood from the two limbs. This indicates that the denervated limb

TABLE 2

Average values for the respiratory quotients of normal and denervated limbs and of whole animal and the arterial-venous oxygen differences

CONDITION OF EXPERIMENT	R.Q.			ARTERIAL-VENOUS OXYGEN DIFFERENCE	
	Normal limb	Denervated limb	Entire animal	Normal limb	Denervated limb
Average of 23 experiments on 13 resting animals	0.77	0.83	0.813	4.22	3.36
Probable error of mean	±0.028	±0.033	±0.004		
Average of 4 experiments on glucose fed animals	0.86	0.92	0.858	3.12	3.97
Probable error of mean	±0.098	±0.125	±0.003		
Average of 5 experiments on exercising animals	0.93	0.96	0.816	5.15	4.12
Probable error of mean	±0.072	±0.077	±0.003		
Average of entire series	0.81	0.86	0.819	5.15	4.12
Probable error of mean	±0.026	±0.029	±0.003		

behaved essentially in a normal way as far as the exchange of sugar was concerned. Lack of information as to the volume of blood flow prevented calculations as to the exact amount of sugar exchange between blood and tissue.

Respiratory quotient. The average values obtained for the respiratory quotient of normal and denervated limbs and the entire animal are given in table 2. The respiratory quotient of a limb was calculated from the differences in the oxygen and carbon dioxide content of arterial and venous blood. In our experiments no attempt was made to correct the carbon dioxide and oxygen content of venous blood for possible changes in concentration and carbon dioxide tension. Corrections for concentration

changes were made in the experiments of Himwich and Castle (1927). In most instances the corrections were within the errors of the analytical methods and did not significantly alter the average values for the respiratory quotient.

The mean respiratory quotient found in 32 experiments on 14 animals was 0.809 ± 0.026 for the normal limb and 0.863 ± 0.029 for the denervated limb. The mean respiratory quotient for the entire animal in the same series was 0.818 ± 0.003. The respiratory quotient of the denervated limb was greater than that of the normal limb in 19 out of 32 experiments. The mean differences are, however, not significant. No difference was noted between the experiments carried out in early or late atrophy. The choice of amytal or procaine anesthesia did not appreciably affect the values for the respiratory quotient. The difference between the oxygen contents of arterial and venous bloods was greater in the control than in the denervated in 20 out of 32 experiments. Blood drawn during the exercising condition showed more than twice the arterial-venous oxygen difference noted in the resting condition.

Discussion. It is to be noted that the results of these experiments are based upon the analysis of blood from the whole limb and not from isolated muscles. It is believed that because of the relatively small metabolism of the non-muscular tissue of a limb, it is permissible to ascribe any significant differences in the composition of arterial and venous blood to the metabolism of muscle. The results of our experiments indicate that skeletal muscle, when deprived of its nerve supply and allowed to undergo the process of atrophy, exhibits a metabolism similar to that of normal intact muscle. Our results confirm those of Himwich and Castle (1926) in that the respiratory quotient of the limb is close to that of the whole animal. Similar to the findings of the above investigators, our results show a wide variation in the individual respiratory quotients of the limbs. These variations are greater than can be ascribed to analytical errors. This represents a marked contrast to the uniform values found for the respiratory quotient of the whole animal. However, it is to be remembered that the latter represents an average of the metabolic activities of the whole animal during a ten minute period and the former represents that of a part over a relatively short time. It is probable that the mixture of foodstuffs oxidized by the tissue varies from moment to moment. When the mean values are considered it is apparent that there is no significant difference between the respiratory quotients of the normal and denervated limbs under the conditions of rest, mild exercise and glucose ingestion. The choice of local or general anesthesia and the time of study after denervation did not have a significant effect on the values for the respiratory quotient. This indicates that skeletal muscle, while undergoing atrophy of denervation, oxidizes a mixture of foodstuffs not significantly

different from that of normal intact muscle or the whole animal. It would appear that the nervous system does not exert a direct control over the particular mixture of foodstuffs oxidized by a muscle. The finding of a greater mean arterial-venous oxygen difference in the normal limb than in the denervated limb is contrary to the observations of Langley and Itogoki (1917) on the anesthetized cat. Although the differences in the sugar and lactic content of arterial and venous blood are small in both normal and denervated limbs, it is evident that in the atrophy of denervation the exchange of lactic acid and sugar between blood and tissue is essentially normal.

SUMMARY

A comparison was made of the sugar, lactic acid, oxygen and carbon dioxide content of blood entering and leaving a denervated limb with that from a normal control limb. The experiments were carried out on dogs under local or amytal anesthesia at periods varying from ½ hour to 14 weeks after denervation. Studies were made during rest, mild exercise, glucose administration and after the subcutaneous injection of adrenalin. It was found that the respiratory quotients (calculated from the difference in CO_2 and O_2 content of arterial and venous blood) of the denervated limbs was essentially the same as that of the normal control limbs and approximated those found for entire animal. A denervated limb resembled a normal limb in the exchange of sugar and lactic acid between the tissue and blood stream. It would appear that the presence of an intact nerve supply does not determine the mixture of foodstuffs oxidized by muscle and that skeletal muscle undergoing atrophy of denervation exhibits a metabolism qualitatively similar to that of normal muscle as far as can be determined by analysis of blood.

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STUDIES ON THE CONDITIONS OF ACTIVITY IN ENDOCRINE ORGANS

XXVII. The Question of Reflex Inhibitory Action of the Vagus on Medulliadrenal Secretion

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Cannon and Rapport (1921) have shown that the reflex center for medulliadrenal secretion lies near the upper or front edge of the floor of the fourth ventricle and that this center can be reflexly inhibited and stimulated. In the hypoglycemia induced by insulin there is stimulation of this center, as observed by Cannon, McIver and Bliss (1924). They also found a prompt subsidence of sympathico-adrenal action on intravenous administration of glucose. The present investigation was undertaken to learn whether afferent vagal influences from the abdomen played a rôle in causing this subsidence.

Richards and Wood (1915) found that the depressor nerve and the left vagus nerve containing depressor fibres from the arch of the aorta inhibited medulliadrenal secretion. Their work was corroborated by Cannon and Rapport (1921). There is evidence that the vagus trunks contain afferent fibres from the stomach (Miller, 1911). Neither Richards and Wood nor Cannon and Rapport tested the abdominal vagal trunk to determine whether or not depressor impulses might originate likewise in this area. It seemed possible not only that influences inhibiting sympathico-adrenal activity might arise in that area, but also that they might travel over afferent vagal pathways. Accordingly the vagi connecting with the splanchnic area were tested by central stimulation to determine whether they contained afferent fibres inhibiting medulliadrenal secretion. Control observations were made by stimulating the left vagus in the neck where fibres inhibiting the secretion are known to be present.

Method. Our observations were all made upon young healthy cats chosen at random. Variations of medulliadrenal secretion were determined by recording the rate of the denervated heart as described by Cannon and Rapport (1921). Persistent secretion of adrenin was induced by making a pseudaffective preparation, as described by Cannon and

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Britton (1925). The decortication permitted observations on animals without anesthesia, the disturbing effects of which are well known. The animals were under light ether anesthesia until decortication and then a period of 15 to 30 minutes was allowed for blowing off the ether. The pseudaffective state usually develops in 5 to 10 minutes after decortication. This preparation has a continuous secretion of adrenin which Cannon and Rapport (1921) showed could be inhibited by stimulation of the depressor nerve or the vagus nerve in the neck.

The technique as described by Cannon and Britton (1925) was modified as follows. The branches of the right (or left) vagus were cut as they left the vagal trunk, from above the recurrent laryngeal to below the bifurcation of the trunk on the esophagus. The trunk itself was isolated along this extent. The other vagus was cut in the neck. The upper thoracic sympathetic chains were removed. The free end of the trimmed vagus was brought out of the chest through the second interspace for stimulation. Great care was taken to prevent injury of it by stretching, drying or pressure during and after its isolation. Decortication was not performed until after completion of all operative procedures, since it was found that animals were unable to tolerate severe surgical measures after being decorticated. Two preparations, nos. 12 and 13, had their hearts aseptically denervated some days earlier, by the method of Cannon, Lewis and Britton (1926), in order to reduce the operative trauma on the day of the acute experiment.

Blood pressure and heart rate were recorded by means of a mercury manometer connected with a carotid artery. The rate was counted before, during and after each stimulation. Changes in heart rate of less than 6 beats per minute were considered to be within the normal range of variation.

A tetanizing current from an inductorium was used throughout. The strength of the current was adjusted to be barely perceptible on the tip of the tongue.

Following is a typical protocol:

No. 27. November 20, 1930. Tiger, female, cat. Weight, 2.8 kgm.

p.m.

1:35 Ether started.

1:40 Tracheal cannula in place. Artificial respiration.

1:45 Thorax opened through left fifth interspace. Left vagus isolated and all branches cut from above recurrent laryngeal to below bifurcation on esophagus. Fine silk thread tied about lower end and nerve cut peripherally. Depressor fibres cut on arch of aorta. Thorax closed.

1:55 Thorax opened through left second interspace. Bilateral sympathectomy, stellate to ganglion at level of third interspace. Vagus drawn through chest and packed in cotton moistened with normal salt solution.

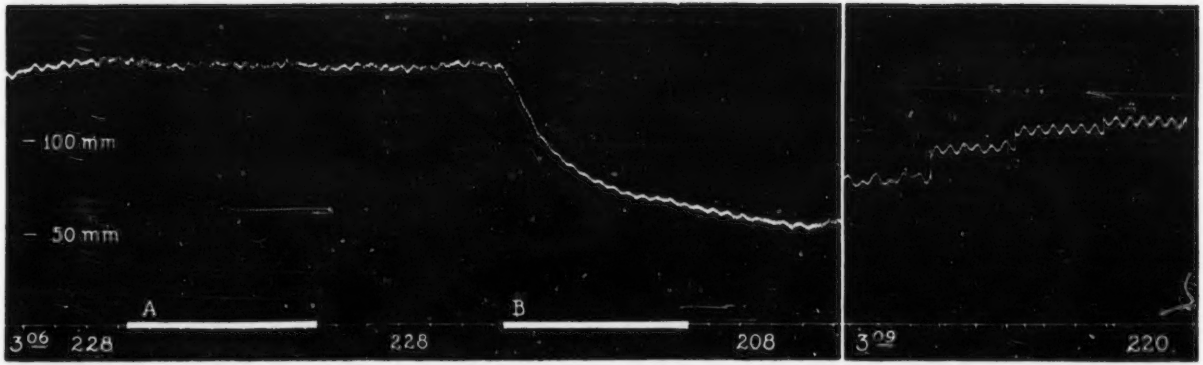

Fig. 1. Experiment 30. March 24, 1931. A. Stimulation of vagus below cardiac and pulmonic branches. No change in heart rate or blood pressure. B. Stimulation of same nerve at the cardiac and pulmonic branches 1 cm. above former site. Same strength of current used. Drop in heart rate from 228 to 208 with return to 220 five minutes later. Fall in blood pressure from 140 to 60 mm. Hg with return to 110 mm. Hg.

2:05 Bilateral carotid ligation. Right vagus cut in the neck. Hemispheres pithed via orbits with manual compression of vertebral arteries for 2 minutes after pithing.
2:10 Right carotid cannula inserted. Rectal thermometer introduced.
2:20 Animal "pseudaffective,"—struggling, and lashing bushy tail. Hind paws wet with sweat. Heart rate, 228. Respiration, 240. Rectal temperature, 39.2°C. Blood pressure, 120 mm. Hg.
2:35 First stimulus.

Post mortem. Sympathetic excised below fourth costal interspace on right and below third interspace on left. Vagus found cut below bifurcation on esophagus. Depressor nerve cut. Anterior portion of cortex ablated with some injury to thalamus and with slight bleeding.

TABLE 1

EXPERIMENT NUMBER	DATE	VAGAL STIMULATION BELOW CARDIAC AND PULMONIC BRANCHES			VAGAL STIMULATION ABOVE CARDIAC AND PULMONIC BRANCHES			VAGUS NERVE STIMULATION
		Before	After	Change	Before	After	Change	
	1930							
10	October 20	236	228	−8	230	212	−18	Left
12*	October 30	207	208	+1	201	192	−9	Left
13*	October 31	249	248	−1	264	252	−12	Left
20	November 13	246	244	−2	234	228	−6	Left
22	November 17	226	222	−4	222	204	−18	Left
24	November 19	224	222	−2	222	212	−10	Right
25	November 19	222	222	0	240	210	−30	Right
26	November 20	220	216	−4	213	198	−15	Right
27	November 20	234	234	0	234	222	−12	Left
	1931							
30	March 24	228	228	0	228	208	−20	Left
Average variation in heart rate per minute.....				−2			−15	

* Previous aseptic denervation of heart.

RESULTS. We were unable to find any inhibition of medulliadrenal secretion, as shown by slowing in the rate of the denervated heart, on central stimulation of either vagus below the region of the heart and lungs. On the other hand, when the stimulus was applied at or above the region of the cardiac or pulmonic branches there was always a definite decrease in heart rate. At the end of stimulation the rate returned to a higher level. The accompanying table gives our results in ten cases. On stimulating the vagus below the heart and lungs the decrease in heart rate averaged in ten experiments 2 beats per minute. On stimulating the vagus above the heart and lungs the decrease in heart rate in the same animals averaged 15 beats per minute. In figure 1 the graph of a typical

result is shown in which there was no change in heart rate or blood pressure on stimulating the vagus below the cardiac and pulmonic branches, but a profound drop on shifting the electrodes 1 cm. higher to the site of origin of the lower cardiac and pulmonic branches.

DISCUSSION. The fact that we were unable to show any definite inhibition of medulliadrenal secretion on central stimulation of either vagus below the heart and lungs agrees with the work of Brodie and Russell (1900). They traced in both vagi the origin of fibres having a depressor action on blood pressure and on the rate of the normally innervated heart and found that the fibres originated chiefly in the lungs. On central stimulation of either vagus below the heart and lungs they observed that no inhibition followed in the cat as a rule, although in the dog slowing occurred in more than half the experiments.

They state, however, that strong currents were necessary, and in the published graph the secondary coil of their inductorium was described as set at 0 cm. In contrast, when they stimulated the vagus fibres to the lungs and obtained a marked and constant fall in blood pressure, the secondary coil was set at 10 cm. It is conceivable that with the very strong currents, spreading occurred and produced inhibitory effects because they reached to the region where pulmonic and cardiac fibres were present.

Likewise our findings are in agreement with those of Cannon, McIver and Bliss (1924) who found that intravenous administration of glucose led to a prompt inhibition of the medulliadrenal hypersecretion accompanying hypoglycemia even though both vagi were cut in the neck.

CONCLUSIONS

Impulses inhibiting medulliadrenal secretion are not carried by the vagi from the splanchnic area.

Adrenal secretion can be inhibited by impulses carried by either vagus from the region of the heart and lungs.

The writers wish to express their appreciation to Dr. W. B. Cannon for suggesting this work and for his helpful criticisms throughout its execution.

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A COMPARISON OF THE EFFECTS OF ACID-BASE CHANGES UPON RESPIRATORY MOVEMENTS AND THE RESPONSE OF MUSCLE TO DIRECT, INDIRECT, AND REFLEX STIMULATION

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In a previous paper (Gay, 1930) it was shown that impaired oxidations produced by low oxygen mixtures, sodium cyanide and sodium sulfide, were accompanied by an increased response of muscle when stimulated directly, indirectly through the motor nerve, and reflexly. It was pointed out that this increased response of muscle so stimulated might operate as an accessory peripheral control of respiration during periods of impaired oxidations. Glazer (1929) has shown that increased alveolar carbon dioxide was accompanied by a decreased reflex response to submaximal stimulation. He has also shown that injection of sodium carbonate was accompanied by an increased reflex response to submaximal stimulation. Winkler (1930) has shown that injection of sodium carbonate was accompanied by an increase in response to submaximal stimulation of the muscle when stimulated directly and when stimulated indirectly through its motor nerve. During supermaximal stimulation under the same condition, however, he found usually no change in response. In the present experiments a more comprehensive study of the changes in response of muscle to various methods of stimulation during changes in acid-base equilibrium has been made.

Various procedures were used for varying the acid-base equilibrium. Gas mixtures of from five to twenty-five per cent carbon dioxide in pure oxygen were administered artificially during pneumothorax and also with the chest intact and the animal breathing naturally. The animal was artificially over-ventilated and under-ventilated in pure oxygen. One-half molecular sodium carbonate solutions were injected in varying amounts. All of these procedures were carried out during both submaximal and supermaximal stimulation. Ventilation and abdominal respiratory movements as well as muscular contractions were recorded. These may be compared in figures 1 to 7.

RESULTS. *Submaximal stimulation.* Figure 4 shows a typical decrease in response of the muscle stimulated through its motor nerve and of the

Figs. 1–7

muscle stimulated reflexly during the administration of carbon dioxide mixtures. In this instance ventilation was artificial. The same decrease took place earlier in the experiment when carbon dioxide was administered naturally. Figures 1 and 3 show typical responses of the muscle stimulated indirectly through its motor nerve and of the muscle stimulated directly during administration of carbon dioxide mixtures. In figure 1 ventilation was natural. In figure 3 ventilation was natural during the first half and artificial during the second half. In each instance decreased responses accompanied the administration. The decreased responses shown in these three records while typical were not without exception. In one experiment administrations of ten and twenty per cent carbon dioxide in oxygen mixtures were accompanied by an increase in response of the muscle stimulated indirectly through its motor nerve during natural ventilation. The muscle directly stimulated showed, at the same time, a decreased response. Later in the same experiment when twenty per cent carbon dioxide in oxygen was administered artificially, there was a decreased response to both indirect and direct stimulation. Whether these exceptions are significant or whether they are due to some slip in the procedure is difficult to say. It is possible that the lowered oxidations may have been more effective in influencing the response of the muscle than was the increased acidity.

Figure 6 shows the response of the muscle directly stimulated and of the muscle stimulated indirectly through its motor nerve during injection of one-half molecular sodium carbonate. In the early part of the figure ventilation was natural; in the latter part ventilation was artificial. The earlier injection shows the typical increased response of the muscle stimulated directly and of the muscle stimulated indirectly through its motor nerve. During the second injection, which was lethal, there was a marked increase in tonus. Muscle reflexly stimulated usually showed the typical increase in response with injection of sodium carbonate.

Figures 2 and 7 show the typical decrease in response of the muscle stimulated indirectly through its motor nerve and reflexly during under-ventilation in pure oxygen. Muscle stimulated directly usually showed the same decrease in response during under-ventilation in pure oxygen.

Figure 5 shows the beginning of one of several experiments in which irregular ventilation occurred, hyperpneas giving way to apneas of varying lengths. It will be seen that periods of decreased response to submaximal indirect and reflex stimulation accompany apneic periods and that periods of active pulmonary ventilation are accompanied by increased response. It may be assumed that apnea leads to decreasing oxidations and increasing accumulation of acid in the blood and tissues and hyperpnea to the reverse. Since impaired oxidations produced by gaseous mixtures low in oxygen have been shown to be associated with increased muscle response to sub-

maximal indirect and reflex stimulation, it is probable that the decreased responses found here are related to increasing acidity and that the increased responses during hyperpnea are related to decreasing acidity.

Figure 1 is of interest in connection with the slow and irregular pulmonary ventilation present at the beginning of the experiment due to some unknown cause. As ventilation increased there occured a concurrent increase in response of the muscle directly stimulated. The increasing pulmonary ventilation augmenting carbon dioxide elimination undoubtedly turned the blood and tissues more alkaline. The increased response to submaximal direct stimulation may therefore be due to increasing alkalinity. Later with an increasing acidity during administration of carbon dioxide respiration underwent a marked increase while the response to submaximal direct stimulation was completely inhibited.

Briefly, the results with submaximal stimulation show that administration of mixtures of carbon dioxide in pure oxygen and under-ventilation with pure oxygen were accompanied by decreased response of the muscle stimulated directly, indirectly, and reflexly; and the injection of sodium carbonate during submaximal stimulation was accompanied by an increase in response of the stimulated muscle.

Supermaximal stimulation. The administration of carbon dioxide mixtures, under-ventilation with pure oxygen, and injection of sodium carbonate were usually accompanied by no change in response to direct, indirect, and reflex stimulation. No records showing supermaximal stimulation are presented. In the few instances when changes did take place they were in the same direction as those occurring when stimulation was submaximal. The change, however, was always very small.

Discussion. As previously pointed out (Gay, 1930) lowered oxidations produced by gaseous mixtures low in oxygen and injection of sodium cyanide and sodium sulfide were accompanied by increased response to submaximal stimulation, direct, indirect, and reflex. The results of the present experiments indicate that increased acidity was accompanied by decreased response to similar stimulation. It would thus appear that during decreased acidity and during impaired oxidations responses to submaximal stimulation, direct, indirect, and reflex, are increased while with increasing acidity responses to the same types of stimulation undergo a decrease. During impaired oxidations, therefore, responses of the stimulated muscle and pulmonary ventilation or the response of the respiratory mechanism proceeded in the same direction. During acid-base changes produced by administration of carbon dioxide or alkaline solutions, however, the response of muscle and of the respiratory mechanism proceeded in the opposite direction.

It has been shown that the increases in response to submaximal stimulation associated with lowered oxidations were absent when the stimulus

was supermaximal. It was, therefore, concluded from these observations that the changes in response with submaximal stimulation accompanying lowered oxidations were due to changes in tissue irritability. The same conclusions may be drawn from the present experiments since changes in acidity were associated with changes in response to submaximal stimulation only. Since there was a decrease in response of the muscle to which submaximal stimuli were directly applied during increased acidity, it may be concluded that increased acidity decreases the irritability of muscle tissue. The same decreased response has been shown to occur in the muscle stimulated through its motor nerve and reflexly through the sensory nerve. The method employed, however, does not serve to show whether this decrease is due to decreased irritability of the muscle alone or whether the nervous tissue involved may not also have undergone some decrease in irritability.

Assuming that the changes in response of the muscles studied are typical for skeletal muscle throughout the organism including the respiratory muscles, these experiments clearly point to a dual accessory peripheral chemical control of pulmonary ventilation inherent in cells outside the respiratory center proper. In the case of lowered oxidations and also with decreasing acidity this peripheral control might tend to augment the effectiveness at the muscle of stimuli originating in the respiratory center. In the case of increasing acidity this peripheral effect might, however, tend to decrease the ultimate effectiveness at the muscle of stimuli which originated in the respiratory center.

SUMMARY

The contractions of the anterior tibialis muscle when stimulated reflexly and indirectly through its motor nerve and of the sartorius muscle when stimulated directly were recorded during administration of mixtures of carbon dioxide in pure oxygen during injection of one-half molecular sodium carbonate and during over- and under-ventilation in pure oxygen.

When submaximal induction shocks were used, there was an increased response to all three types of stimulation during administration of sodium carbonate and a decreased response during administration of carbon dioxide rich mixtures and during under-ventilation in oxygen. When supermaximal stimuli were used, the changes in response were missing or small.

Since the response of muscle to submaximal stimuli applied to the muscle directly was decreased during periods of increased acidity, it was concluded that the irritability of muscle tissue was decreased by increasing acidity. Since the effect of a stimulus applied to the nerve whether motor or sensory is, by the method employed, expressed as a muscular response and since the muscle is itself affected by acid changes, no conclusions have been made as to the effect of increasing acidity upon irritability of nervous tissue.

The parallelism of the response of muscle to submaximal stimulation,

direct, indirect, and reflex, and of pulmonary ventilation during periods of impaired oxidations produced by administration of oxygen poor mixtures is contrasted with the inverse relation of the responses of the muscle and of pulmonary ventilation during acid-base changes. It has been suggested that this parallelism between the response of pulmonary ventilation and muscle to lowered oxidations might tend to enhance the effectiveness at the muscle of stimuli originating in the respiratory center and that the inverse relation of the response of pulmonary ventilation and muscle to acid-base changes might tend to decrease the effectiveness at the muscle of stimuli originating in the respiratory center.

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CONDITIONS GOVERNING THE REMOVAL OF PROTEIN DEPOSITED IN THE SUBCUTANEOUS TISSUES OF THE DOG

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Since Starling's work in 1894, the capillaries of the body, with the exception of those of the liver and intestinal mucous membrane, have been considered to be wholly impermeable or at best only slightly permeable to protein.

Churchill, Nakazawa and Drinker (1927) believed that the skin capillaries of the frog were normally permeable to protein, since the fluid in the lymph spaces always contained from 0.29 to 2.7 per cent of protein. Conklin (1930) extended these observations on the skin capillaries of the frog and showed that foreign proteins, with molecules of varying sizes, injected into the circulation, passed easily through the skin capillaries and could be recovered in the lymph in a very short time. In no case, however, was she able to find any absorption by the blood of colloids injected into the lymph sacs. She suggested that pressure and osmotic relations, both of which normally oppose return to the blood, might account for the failure of protein to pass from the tissue spaces back through the capillary endothelium.

Working on the dog, Drinker and Field (1931) and Field and Drinker (1931) were able to demonstrate a graded though generalized permeability to protein on the part of the blood capillaries in different parts of the body, but were unable to obtain any evidence of the passage of protein from the subcutaneous tissues back through the capillary endothelium in dogs whose lymphatic entrances into the blood had been completely obstructed. Batchelder, Field and Drinker (1931) found a slight degree of vascular absorption of nickel-containing particles by the omental vessels. With the possible exception of this region, it seems probable that protein which has left the blood vessels can be returned to the blood via the lymphatic route alone when conditions in the animal are normal.

Hirota (1928), in his work on the restitution of the blood fluid after hemorrhage, found that in every case the colloid osmotic pressure of the blood plasma was quickly though not completely restored after hemor-

rhage. As a possible explanation, he suggested the direct transfer of lymph protein into the blood through the capillary endothelium.

Morawitz (1916) found that dogs whose blood protein content had been reduced by hemorrhage to about 2 per cent were able to restore from 2 to 3 per cent of the protein during the first hour. The albumin was restored much sooner and more readily than the globulin. He suggested that tissue fluid and lymph might contain the more easily filtered albumin in abundance rather than globulin, and that the organism draws into the blood a protein fluid rich in albumin in its attempt to restore the lost fluid. This suggestion is in accordance with results obtained by Loewen, Field and Drinker (1931).

The purpose of the following experiments was to determine whether or not the passage of protein in one direction only—through the capillary endothelium to the tissues but not vice versa—could be explained by pressure and osmotic relations. Under normal conditions the capillary pressure exceeds the osmotic pressure of the plasma colloids, whose effectiveness is further reduced by the presence of a proteinized tissue fluid, thus making the pressure gradient toward rather than from the tissues. Since the concentration of protein in the blood is considerably higher than that of the tissue fluid, simple diffusion of protein through the capillary endothelial wall would be prevented. It was thought that by reversing these conditions protein placed in the subcutaneous tissues might be able to pass back through the capillary wall into the blood stream.

EXPERIMENTAL PROCEDURE. Dogs were employed in this series of experiments. They were anesthetized with Nembutal "844" (sodium-ethyl (1-methyl-butyl) barbiturate)[1] which was given intraperitoneally in a dose of 40 mgm. per kilo. The lymphatic entrances into the blood at the venous confluence on both the right and left sides of the neck were carefully isolated and ligatured. Cannulas were inserted into the right and left cervical lymphatic ducts higher in the neck and into the thoracic duct, and collections of lymph were made. The blood pressure was taken from either the carotid or femoral artery and reinjections were made through the femoral vein. From 800 to 1000 cc. of blood were removed. Washed dog blood corpuscles suspended in physiological saline or in Locke's solution were immediately reinjected. Normal sterile horse serum with a protein content of 6.9 per cent determined refractometrically was infiltrated by multiple punctures under the skin of the neck, thorax and abdomen, and its presence in serum and lymph was determined by means of anti-horse rabbit serum.

RESULTS. The results of three typical experiments and a single control

[1] The authors wish to thank Dr. J. F. Biehn of the Abbott Laboratories for his kindness in furnishing a supply of this excellent new anesthetic.

are embodied in table 1. Numerous further control experiments are given in a previous paper (Field and Drinker, 1931).

Discussion. Under normal conditions protein which has left the blood vessels or which has been placed in the subcutaneous areas is unable to pass back through the capillary endothelium but must return to the blood stream by the lymphatic route.

After plasmapheresis the situation is quite different, as table 1 shows. The blood pressure is temporarily reduced and the protein concentration

TABLE 1

Rate of appearance of subcutaneously injected horse serum in blood, cervical lymph, and thoracic duct lymph following plasmapheresis

	EXPERIMENT 1		EXPERIMENT 2		EXPERIMENT 3		EXPERIMENT 4 (CONTROL)	
Per cent of protein in dog serum before plasmapheresis	7.24		6.55		7.42		8.36	
Time and amount of subcutaneous injection of horse serum (6.9 per cent protein)	1:28 p.m. 15 cc.		2:55 p.m. 30 cc.		12:41 p.m. 35 cc.		11:25 a.m. 30 cc.	
Plasmapheresis. Cubic centimeters of dog plasma removed	700 cc.		500 cc.		500 cc.			
Per cent of protein in dog serum	3.68		2.25		3.17		8.36	
Duration of experiment	1½ hours		1¼ hours		3 hours		5 hours	
	Time	Amount	Time	Amount	Time	Amount	Time	Amount
Appearance of protein in blood	2:00	−	3:12	−	12:56	±	11:55	−
	2:30	+	3:27	+	2:40	+	1:25	−
	3:00	+	4:12	++	3:50	++	4:25	−
Appearance of protein in cervical lymph	2:00	−	3:12	−	1:10	+	11:55	±
	2:30	−	3:27	−	1:40	++	12:25	++
	3:00	−	4:12	−	3:10	±	4:25	++
Appearance of protein in thoracic duct lymph	2:30	−	3:12	±	1:10	−		
			3:27	−	1:40	+		
			4:12	+	3:10	±		

of the blood is lowered to around 3 per cent. The restoration of the blood volume is accomplished by withdrawal of fluid from the tissues, and foreign protein infiltrated in the subcutaneous tissues together with water and salts is taken up by the blood directly.

The capillary endothelium is thus permeable to protein in both directions, although, under normal pressure and osmotic relations, protein which has left the blood is returned by the lymphatic route alone.

SUMMARY

1. The capillaries under normal conditions are not concerned with the absorption of protein from the subcutaneous tissues.

2. After plasmapheresis, with substantial reduction of total blood protein, foreign protein placed in the subcutaneous tissues can be detected serologically in the blood when entrance by lymphatic routes has been blocked.

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THE COLLOID OSMOTIC PRESSURE OF DOG BLOOD AND LYMPH

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In 1927, Churchill, Nakazawa and Drinker (1927) measured the colloid osmotic pressure of lymph and blood taken from frogs. The direct method of Krogh (1924; also Krogh and Nakazawa, 1927) was used. The average osmotic pressure for the lymph proteins was found to be 42 mm. of water, that of the blood proteins in the same animals 71 mm. of water. It was pointed out that the "effective colloid osmotic pressure" for restoring water to the blood capillaries was the difference between these figures—29 mm. of water. Operating against this pressure was the capillary blood pressure of the frog—around 130 mm. of water, which must assure a rapid filtration of water through the capillary walls.

In recent papers, two of the authors (Drinker and Field, 1931; Field and Drinker, 1931) have given figures for the protein content of dog lymph, together with experimental data, which have led to the conclusion that the lymph and the tissue fluid in which the body cells live are approximately identical in composition. It follows that measurements of the osmotic pressure of blood and lymph proteins, taken simultaneously, must be of interest in indicating some measure of the actual forces bringing about movements of water from and into mammalian blood capillaries.

METHODS. Dogs anesthetized with sodium barbital given intravenously were used. Lymph was collected from cervical lymphatics and from the thoracic duct as described in previous papers (Drinker and Field, 1931; Field and Drinker, 1931).

To determine the colloid osmotic pressure the first or small tube method of Krogh (1924; also Krogh and Nakazawa, 1927) was employed. The collodion membranes used corresponded to the group described by Krogh as 4B. In 24-hour tests these membranes permit the passage of an extremely small trace of protein. The preparation and testing of such membranes is not easy. Our experience with different specimens of collodion indicates that in every instance the procedure employed will vary somewhat from the original descriptions, but that with care one can obtain membranes of the required permeability to water and protein. As outside fluid, Ringer's solution of the following compositon was used:

	gm.
NaCl	0.9
KCl	0.042
$CaCl_2$	0.024
$NaHCO_3$	0.01
Glucose	0.1
Water	100 cc.

The results obtained by dialyzing against this fluid were checked by using protein-free ultrafiltrates from dog lymph. The differences in results were insignificant, varying both above and below the values obtained with the Ringer solution.

TABLE 1

Protein content and colloid osmotic pressure of blood and lymph from dogs

DOG	BLOOD SERUM			CERVICAL LYMPH			THORACIC DUCT LYMPH		
	Protein	Osmotic pressure	Osmotic pressure per gram of protein	Protein	Osmotic pressure	Osmotic pressure per gram of protein	Protein	Osmotic pressure	Osmotic pressure per gram of protein
	per cent	*mm. H_2O*	*mm. H_2O*	*per cent*	*mm. H_2O*	*mm. H_2O*	*per cent*	*mm. H_2O*	*mm. H_2O*
1	8.78			3.38	187	55	4.82	286	59
2	8.84	412	59	2.37	172	73	4.93*	344	70
							4.47†	336	74
3	8.02	371	46	1.67	131	78			
4	8.36	366	44				3.24	214	66
5	7.20	334	46	1.73	160	92	3.17	225	71
6	7.50	466	60	1.87	142	76			
7	4.19			1.48			2.68	181	67
8	7.91	350	44	2.94	195	66	3.52	288	64
9	6.98	340	49	1.68	173	103	2.12	138	65

* First.
† Last.

Blood and lymph protein percentages were estimated refractometrically.

EXPERIMENTS. The results obtained are shown in table 1.

DISCUSSION. Table 2 summarizes findings upon the colloid osmotic pressure of mammalian blood serum. It becomes clear that the values for dog serum presented in table 1 are within the range of expectation, and since the figures given for lymph have been obtained in an exactly similar manner, they undoubtedly represent a reasonable approximation of the absolute values.

Certain interesting points may be made, using the data from table 1. First of all, if lymph represents the true watery environment of the body cells, as the authors believe to be the case, then the "effective osmotic

pressure" of the blood proteins is the colloid osmotic pressure of the blood serum minus the colloid osmotic pressure of lymph from the drainage area under observation. In dog 2, this would mean 412 − 172 = 240 mm. of H_2O for the cervical region. Thoracic duct lymph comes in the main from the abdominal organs, particularly the intestines. In our experience the protein concentration of this lymph is occasionally almost equalled by that of normal subcutaneous lymph, and twice has been exceeded. The high protein content of thoracic duct lymph is an expression of the great permeability of intestinal and liver capillaries, and in these regions the "effective osmotic pressure" for drawing water into the blood vessels must be extremely low.

TABLE 2

Osmotic pressure of mammalian blood proteins in serum or plasma

AUTHOR	PROTEIN	NUMBER OF ANIMALS AND SPECIES	OSMOTIC PRESSURE
	per cent		*mm. H_2O*
Landis (1930)		Rat	220–265
		Guinea pig	220–270
Krogh (1929)	4.8–6.2	7 rabbits	230–330
Fishberg (1929)	5.7–7.2	3 rabbits	234–350
Johansen (1930)	7.2	Rabbit	322
Hirota (1928)		Horse	250–340
Krogh and Nakazawa (1927)		Horse	221–274
Govaerts (1924)	7.67–8.69	10 men	357–397
Dieter (1925)		54 men and women	370–480
Runge and Kessler (1925)		22 women	302–335
Mayrs (1925–1926)	7.87	Man	402
Verney (1926)	7.48	Man	350–367
Iversen and Nakazawa (1927)	7.2–8.6	8 men	325–401
Cope (1928–1929)		4 men	309–320
Ito, Seki and Nakazawa (1929–1930)	6.9–10.0	34 men	315–465
Strandqvist (1930)	6.4–9.14	24 men and women	295–426
Rabinowitch (1930)	5.16–8.31	32 men and women (diabetic)	242–497
Schade and Claussen (1924)		10 men	289–372

The second matter for notice in table 1 is the unvarying excess of osmotic pressure per gram of protein which is possessed by both cervical and thoracic duct lymph over blood. It seems probable that this excess pressure is the expression of the greater permeability of the blood capillaries for serum albumin, which has a molecular weight about one-half that of serum globulin (Adair and Robinson, 1930) and a correspondingly greater osmotic pressure. Attempts to separate albumin and globulin with thorough accuracy in small amounts of lymph have not been successful. The only separations we have found in the literature are the work of Munk and Rosenstein (1891)

and Morawitz (1906) for thoracic duct lymph. These determinations are so variable as to provide nothing except confusion.

In view of the greater permeability of the blood vessels through which practically all of the thoracic duct lymph must pass, it is interesting to note that per gram of protein this lymph, in all cases except the first and eighth in which the protein of the cervical lymph is very high, is lower than that of the cervical lymph.

SUMMARY

1. The colloid osmotic pressure of the blood of dogs varies between 334 and 466 mm. of water, of cervical lymph between 131 and 195 mm. of water, of thoracic duct lymph between 138 and 344 mm. of water.

2. The colloid osmotic pressure of lymph per gram of protein is higher than blood. The probabilities are that this is due to a higher proportion of serum albumin in lymph than in blood.

3. The "effective osmotic pressure" for returning water to the blood capillaries is the difference between the colloid osmotic pressure of the blood and the lymph from the area under observation.

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SUBSTITUTION OF "LECITHIN" FOR RAW PANCREAS IN THE DIET OF THE DEPANCREATIZED DOG

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The impaired assimilation of protein and fat in the depancreatized dog, as evidenced by the character of the feces, became apparent soon after the production of experimental diabetes by Mehring and Minkowski (1889). The beneficial effect on the digestive powers of partially depancreatized dogs, produced by the addition of raw pancreas to their diet (Sandmeyer, 1895), seemed to indicate that the presence of the digestive enzymes of the pancreas in the intestinal tract was one of the essential factors concerned in the survival of such animals. This belief in the indispensability of the external pancreatic secretions has been further supported since the discovery of insulin by Banting and Best. Thus, various workers have reported that depancreatized dogs did not survive more than from 1 to 8 months on a meat and sugar diet, in spite of complete control of diabetic manifestations by the use of insulin, unless raw pancreas was also provided (Allan, Bowie, Macleod and Robinson, 1924; Hédon, 1927; Fisher, 1923; Bliss, 1922).

While there can be no doubt that raw pancreas improves the digestion of protein and fat in the depancreatized dog, it is not established that this improvement per se is a vital factor in the survival of the animal. Given an adequate protein intake, the utilization of protein by depancreatized dogs not receiving raw pancreas is not excessively low, as judged by urinary nitrogen excretion. As regards the utilization of fat, Abelman in Minkowski's laboratory (1890) showed that a large part of the fat in the feces of these animals appears in the form of fatty acids similar to those produced by the action of pancreatic juice on neutral fat. Whether this is due to bacterial action, as Rosenberg (1898) suggested, or to the action of lipase which has been shown to be present in intestinal juice (Leathes and Raper, 1925) or both, is undecided. According to Bloor (1925), the absence of pancreatic lipase appears to have no great effect on the hydrolysis of fat in the intestine. In any case it seems improbable that lack of digestion of the fat is responsible for the difficulties encountered. These considerations suggest that the beneficial effect of raw pancreas in the diet

of depancreatized dogs does not depend on its content of digestive enzymes. Such a conclusion is supported by the work of Penau and Simonnet (1926), who were able to keep a depancreatized dog, receiving insulin but no raw pancreas or pancreatic enzymes, alive and well for over two years. If, as seems probable from the above, the absence of the pancreatic digestive enzymes is not responsible for the death of depancreatized dogs treated with insulin, the identity of the factor in raw pancreas which is essential to the life of these animals becomes a matter of great interest.

The signs and symptoms of these animals have been well described by Allan, Bowie, Macleod and Robinson (1924):

> Following depancreatization the dogs were placed on diets of from 600–900 grams of lean, raw meat and 100 grams of cane sugar. Glycosuria was present, the degree of which varied according to the dose of insulin. The animals remained in good condition for varying periods of from one to eight months, when in contrast to their previous good behavior, they appeared depressed and apathetic, and took little food. Jaundice was sometimes quite marked and bile pigments were found in the urine, the volume of which became less and less. Vomiting was frequently present. Extreme weakness and diarrhea preceded death, the feces being very dark and occasionally contained small amounts of blood. Post-mortem examination revealed that the liver was the chief and in most cases the only organ obviously affected, marked fatty infiltration (up to 35.5%) being observed.

The belief that the liver is the seat of the major disturbances in these animals is supported by the observations of other investigators. In one of Fisher's experiments insulin was not administered several days prior to the death of the animal, because the urine remained sugar-free. This animal died in hypoglycemic convulsions with a blood sugar level of 0.013 per cent. Convulsions also preceded death in another case reported by the same author. In accord with these results are those of Hédon, who found that some time after the withdrawal of pancreas from the diet of his animals, they became very sensitive to insulin, the dose of which had to be reduced in order to prevent dangerous hypoglycemia. These signs of failure of liver function, coupled as they are with post-mortem evidence of pathological changes confined almost solely to this organ, suggest a hepatic disturbance due to the absence of the pancreas, rather than a general toxic action such as might be attributed to the absorption of products of incomplete protein digestion. The state of the liver at post-mortem examination indicates a disturbance in fat metabolism particularly. These observations agree with those of Lombroso (1909) who, as the result of an extensive investigation, suggested that the pancreas influences the assimilation of fat by means of an internal as well as an external secretion.

It is not within the scope of this paper to review in detail the present knowledge concerning the relation of the liver to fat metabolism. Most

investigators agree that this relationship is a very important one. It is generally accepted that fat is transported to the liver to be desaturated and prepared for use in the body (Leathes and Meyer-Wedell, 1909; Joannovics and Pick, 1910). It is also accepted that the phospholipins occupy an important position in fat metabolism. Whether they are formed in the liver (Kennaway and Leathes, 1909), or in the blood corpuscles (Bloor, 1916) is uncertain and their significance has been variously interpreted. They have been regarded both as an intermediate step in the metabolism of fat (Bloor, 1916), and as the end product of the desaturation process, i.e., "the metabolic form of fat" (Allen, 1922). Some investigators have gone further and have attributed a more active function to the phospholipins. MacLean and MacLean (1927) and Bloor (1925), for example, believe that the available evidence indicates that these substances are concerned in the transport and mobilization of fat for utilization by the tissues. Leathes (1909), on the other hand, has suggested that the lipins play some part in the desaturation of fatty acids in the liver.

In seeking for an explanation of the disturbance in the liver-fat metabolism described above it seemed a reasonable hypothesis that the failure observed might be one involving the desaturation of fat. If this were so, in view of the above suggestion of Leathes, the presence of phospholipins in the organism might be a very important matter. The work which we are reporting originated in 1927 from the ideas of one of us (J. M. H.) that the symptoms of these animals were produced by failure of fat metabolism in the liver, and that lecithin might be substituted for pancreas in the diet. With these points in mind experiments were instituted in which depancreatized dogs, after developing the typical "liver failure" syndrome on a meat-sugar diet with insulin were given "lecithin" in addition to their previous diet. A preliminary report of this work has already been made (Hershey, 1930). The results of several experiments are presented in the accompanying protocols.

METHODS. Complete removal of the pancreas was performed at one operation under ether anesthesia. The day following the operation insulin administration was begun. Lean minced beef muscle and sugar were given in small amounts which were gradually increased on succeeding days until within a week the animal was on full diet. The "lecithin" used was prepared from egg yolk by the Digestive Ferment Co. The Shaffer-Hartmann (1920) method was used for blood and urinary sugar. The Leathes and Raper (1925) modification of the Liebermann method was used for estimation of liver fat. Iodine numbers of the fats were determined by the procedure recommended by Leathes and Raper (1925).

EXPERIMENTAL RESULTS. The course of some of the typical experiments is outlined in the following protocols.

Dog III. Fox terrier, female, weight 7 kgm.

1927

September 19. Completely depancreatized. Diet 150 grams lean beef muscle, 40 grams sugar, 15 units insulin twice daily.

October 7. Urine and feces progressively darker in color.

October 8. Urine positive for bile.

October 14. Urine very dark, feces quite black. Animal distinctly jaundiced.

October 16. Five grams "lecithin" added to each meal.

October 19. Urine and feces almost normal in appearance. Urine negative for bile. "Lecithin" discontinued.

October 26. Animal showed slight insulin reaction, 5 grams "lecithin" added to each meal.

October 27. Animal quite active. Feces soft, orange-yellow in color.

November 1. "Lecithin" discontinued. Sugar in diet increased from 40 to 60 grams each meal.

November 15. Urine progressively darker in color and reduced in volume. Hypoglycemic symptoms on this day. Five grams "lecithin" added to each meal. Also additional amounts of glucose to protect against hypoglycemia were given.

November 26. Weight 6.5 kgm. During the first part of December excessively fat meat led to diarrhea. Substitution of leaner meat restored the animal to excellent health.

1928

January 2. Condition good, but slight suggestion of a skin irritation.

January 3. "Lecithin" discontinued.

January 8–12. Skin condition much worse in spite of addition of orange juice to diet. Animal somewhat listless.

January 13. At 8:30 a.m. the dog was found in violent convulsions which did not respond to the administration of either sugar, calcium or parathormone. Convulsions continued throughout day with blood sugars ranging from 0.151 to 0.098 mgm. per cent and blood calcium from 13.5 (before administration of parathormone) to 12.2 mgm. per cent (the following day).

January 14. Some signs of improvement at 3:30 a.m. At 9:30 a.m. took food and water and from this on ate ravenously and showed extreme thirst. Cod liver oil and calcium lactate given with food.

January 15–16. Considerable red blood in feces.

January 20. Condition has progressively improved. Appetite excellent. Feces practically normal. Skin condition after a slight remission again bad.

February 1. The animal's hind limbs were observed to be stiff. It had difficulty in scratching itself and in stepping over an obstacle. At the same time the animal, previously very noisy, seemed to have lost its ability to bark. It was frequently observed going through the motions of barking with the head thrust forward and neck stretched, but no sound was heard. Five grams "lecithin" added to each meal, also two to three cakes yeast (Fleischmann's) per day.

February 7. Condition stationary.

February 10. Fresh dried brewer's yeast substituted for the commercial variety.

February 21. General condition had improved considerably. Dog active. Any cessation of the administration of fresh brewer's yeast was followed by a progressive decline in general condition.

March 19.	Adult doses of "marmite" substituted for yeast. Skin condition improving.
April 11.	Animal in excellent condition. History uneventful on diet supplemented by "lecithin" and "marmite." Definite indication that voice was returning. The bark was weak and high-pitched.
April 16–18.	Added 10 grams fat (beef suet) to each meal. Analysis of feces showed 50 per cent absorption of fat from the intestine. Urine positive for bile on April 18.
May 2.	Weight 7.9 kgm. Dog in good condition.
May 18.	Condition stationary, though slight weakness of hind limbs observed. Voice improving, bark stronger and more frequent.
June 2.	Hind legs weak and constantly semiflexed with joints stiff.
June 10.	Condition very poor. Eyelids stuck together with thick clear discharge. Vision almost gone. Hind limbs very weak. Cod liver oil administered.
June 13.	Eyes normal. General condition very much improved. Cod liver oil continued, "lecithin" discontinued.
July 1.	Animal in excellent condition. Skin irritation entirely alleviated. Voice normal and animal as noisy as it was originally.
September 19.	History uneventful. Dog in excellent condition, very active.
October 3.	Dog guillotined for post-mortem examination. Post-mortem examination showed no pancreatic tissue on microscopic examination of the intestine. Liver fat content 2.74 per cent—Iodine number of liver fat 152. Liver glycogen content 9.60 per cent—Muscle glycogen content 1.15 per cent.

Dog IV. Collie, female, weight 8.2 kgm.

1927	
September 23.	Completely depancreatized. Diet 150 grams meat, 40 grams cane sugar, 12 units insulin twice daily.
October 15.	Urine progressively darker in color, positive for bile.
October 23.	Definite weakness about 8:00 p.m.
October 24.	Convulsions 8:00 p.m. Blood sugar 0.043 mgm. per cent. Promptly recovered with glucose by stomach tube.
October 25.	Insulin reduced to 8 units twice daily. Convulsions 8:30 p.m. Blood sugar 0.060 mgm. per cent. Promptly recovered with glucose.
October 26.	Insulin reduced to 6 units twice daily. Definite weakness about 8:00 p.m. but no convulsions. Glucose given.
October 27.	Showed weakness at 12:00 noon, convulsions about 8:00 p.m. Recovered with glucose.
October 28.	Repetition of the 27th of October.
October 31.	Meat in diet increased from 150 to 175 grams twice daily.
November 1.	Eight units instead of 6 units with evening meal caused definite weakness during the evening.
November 3.	Urine progressively darker in color and reduced in volume, positive for bile. Feces very dark.
November 8.	Urine very dark, positive for bile, greatly reduced in volume, feces black, animal listless.
November 9.	Bile in urine positive, 5 grams "lecithin" added to each meal.
November 13.	Urine slightly increased in volume, lighter in color. Dog more active.

December 6. Dog in excellent condition, weight 9.2 kgm. Urine normal in color and volume. "Lecithin" discontinued in the diet.

December 9–13. On account of an undue amount of fat in the meat, during these days, the animal developed a severe diarrhea, vomiting, loss of appetite and general apathy.

December 13. Lean meat again used and the above condition disappeared.

December 15. "Lecithin" added to diet.

1928

January 2. Dog in excellent condition, weight 8.3 kgm. "Lecithin" discontinued in the diet.

January 20. Since the previous date it has been necessary to reduce the insulin from 8 to 6 units to prevent reactions. The urine has become progressively reduced in volume and darker in color and positive for bile.

January 21. "Lecithin" added to diet.

January 31. Urine has progressively increased in volume, lighter in color, bile-free. Dog more active.

February 8. Insulin increased from 6 to 8 units twice daily.

February 16–18. Ten grams fat (dripping) given with each meal. Analysis of feces showed an absorption of 30 per cent from the intestine. During this period urine darker in color, reduced in volume and positive for bile.

May 2. Since the above period of fat feeding, urine has increased in volume and become bile-free. Dog in excellent condition. During part of July the dog again showed a decline from its previous good condition, some loss of appetite, and the usual excretory symptoms, due to an increase in the fat content of the food without our knowledge. On the substitution of lean meat and some additional "lecithin" the dog promptly recovered.

August 1. At this time it was noticed that the dog was unable to bark, otherwise in excellent condition.

October 20. Dog in excellent condition, weight 8.8 kgm. Discontinued "lecithin" and substituted 10 grams suet and liberal doses (adult human) of irradiated ergosterol (oscodal) and yeast extract (marmite). The animal progressively lost weight and showed decline in its general condition until its death.

December 19. The urine became progressively decreased in volume, darker in color and positive for bile. Feces became very dark to begin with, gradually looser with a bloody diarrhea preceding death. Loss of appetite was marked. The animal refused to eat for two or three days during which time it apparently improved somewhat in condition. Any food which was eaten affected it in a most unfavorable manner. The suet, "oscodal" and "marmite" with sugar were given twice daily when the animal failed to eat. When the animal's appetite failed, the insulin dosage was reduced to 4 units twice daily to avoid reactions. The dog became suddenly weak before it died and showed an extreme degree of jaundice. Administration of sugar failed to have any appreciable recuperative effect. It was interesting to note that about five weeks after the commencement of the vitamin administration, the animal recovered some ability to bark. Post-mortem examination showed liver content of 22.0 per cent fat. Iodine number of liver fat 67.

December 19. No pancreatic tissue was found on microscopic examination of the duodenal region.

Dog V. Mongrel spaniel, female, weight 5.8 kgm.

1927

November 22. Completely depancreatized, diet 150 grams meat, 30 grams cane sugar, 5 units insulin twice daily.

December 13. Dog has shown progressive loss of appetite and weakness. Weight 4.3 kgm.

December 14. Lean meat substituted for rather fatty meat previously given. Appetite immediately improved and animal quite active.

December 18. Fat meat again allowed in diet. Appetite began to fail.

December 20. Bile in the urine.

December 21. Dog refused to eat, and appeared listless. Ten grams "lecithin" by stomach tube, 10 grams "lecithin" subcutaneously.

December 22. Animal much more active.

December 24. Lean meat supplied.

1928

January 9. Animal becoming less active, urine darker and more concentrated.

January 10. Twenty-four hours' sugar excretion 13.7 grams.

January 11. Twenty-four hours' sugar excretion 8.0 grams.

January 12. Twenty-four hours' sugar excretion 4.4 grams. The collection of urines was at this point unfortunately interrupted.

January 18. Marked hypoglycemic symptoms. Promptly recovered with glucose by stomach tube.

January 19. Vomited evening meal. Convulsions 7:00 p.m. Recovered immediately with glucose.

January 20. Animal very apathetic. Refused evening meal. Blood in feces. Offered a meal of cooked beef and "lecithin" which it ate greedily.

January 21. Regular diet plus 5 grams "lecithin" with each meal.

January 22. Dog very active, appetite excellent, urine lighter in color and increased in volume.

March 8. "Lecithin" discontinued. Ten grams fat (dripping) substituted in each meal. Analysis of feces for two four-day periods showed 80.0 per cent fat absorption from the intestine.

March 18. Urine becoming progressively reduced in volume, darker in color. Feces also darker.

March 24. Fat discontinued, "lecithin" substituted.

March 25. Twenty-four hour sugar excretion followed on regular diet.

March 26. Sugar excretion 6.2 grams.

March 27. Sugar excretion 9.4 grams.

March 28. Sugar excretion 14.8 grams.

March 29. Sugar excretion 18.1 grams. Urine and feces progressively lighter in color. "Lecithin" continued in diet for remainder of experiment. Animal in excellent condition throughout and history uneventful until termination of experiment October 3, 1928. Weight 5.8 kgm. Dog guillotined on this date. Post-mortem examination showed pancreatic tissue "about the size of a split pea," no islet tissue present. Liver fat content 3.75 per cent—Iodine number of liver fat 118. Liver glycogen content 7.90 per cent. Muscle glycogen content 1.40 per cent.

Dog VII. Collie, weight 7.8 kgm.

1928

November 9. Depancreatized. Diet 200 grams meat, 50 grams cane sugar, 6 units insulin twice daily.

April 10–29. Weight 6.5 kgm.

April 18–30. Average daily urinary sugar excretion 2.3 grams, average volume 150–175 cc.

May 23. Vitamins A, B, C and D added to the diet in the form of a tested concentrate for A and D, kindly supplied by Ayerst, McKenna and Harrison, and "marmite" and orange juice for B and C.

May 23–29. Average daily urinary sugar excretion 0.3 gram.

June 14–16. Urinary sugar excretion 1.0 gram. Vitamins dropped from the diet.

August 15–30. Loss of appetite and diarrhea found to be due to excessively fatty meat. Recovered when lean meat fed.

September 9–11. Urinary sugar excretion 0.3 gram.

September 11–13. Urinary sugar excretion 1.2 gram. At this time the catheter urine appeared to be much more concentrated.

October 10. Bile pigments quite positive in the urine.

October 22–25. Average daily urinary sugar excretion 0.5 gram, average volume 160 cc. Bile pigments positive in catheter urine for some time and dog not as active as formerly, requiring careful watching.

October 24–25. Fifteen grams "lecithin" added to the diet.

Oct. 26 to Nov. 16. Five grams "lecithin" each meal, continued in the diet. Average daily sugar excretion for this period of 22 days was 19.6 grams. Urine became bile-free and increased in volume 350–700 cc. daily.

November 17. "Lecithin" dropped from diet.

December 16–20. Average daily sugar excretion has slowly fallen from the previous high values to a daily average of approximately 4.0 grams.

1929

January 9. The dog first refused its food, but during the day ate a very small amount. Not as bright as usual. Insulin dose 2–3 units.

January 10. Dog refused all food. Was allowed out of the cage. Appeared to be very frightened and easily angered. Walked slowly as with an effort, the hind-quarters appearing to be stiff. Vitamin B deficiency or hypoglycemia was suspected. At 5:30 p.m. the dog went into violent convulsions and nearly expired. The blood sugar taken shortly after the onset of convulsions was 0.410 mgm. per cent. Convulsions continued in a violent form with few intervals until 1:30 a.m. when the dog died. Liver fat 18 per cent.

In the original experiment in this investigation it was found that a diabetic animal which has been receiving saturated fats (beef suet) was considerably improved by the substitution of "lecithin" for these fats. This experiment was complicated by the appearance of symptoms of vitamin deficiencies. The "lecithin" had no effect on these symptoms but it appeared to prevent the development of the fatty infiltration of the liver since the liver fat at autopsy was 2.6 per cent with an iodine number of 120. In the second experiment about five weeks after pancreatectomy bile appeared in the urine, and the sugar output was reduced. The administration of "lecithin" resulted in an increased sugar output, disappearance of bile and

an improvement in the general condition of the animal. These experiments are not described in detail since the important points are convincingly demonstrated in the more adequately controlled experiments, the results of which are described in the above protocols.

Discussion. The typical condition, which we believe is largely attributable to a disturbance of liver function, may appear in dogs under the conditions of our experiments as soon as 6 weeks after pancreatectomy. It may not appear until 11 months after the operation. The signs have been discussed in the introduction. The autopsy findings in dogs in this condition have been reported by Allan, Bowie, Macleod and Robinson. We have found, in confirmation of these workers, that the onset of this condition may be accelerated by the addition of saturated fats to the diet.

The beneficial effect of "lecithin" on these animals as indicated by the relief of the signs and symptoms which may have developed, is illustrated in all the protocols. The fact that these animals may survive in good condition for at least a year if "lecithin" is provided, is established by experiments 3, 4 and 5. These animals had exhibited signs of failure of liver function at various times when "lecithin" was omitted and in every case their condition was improved by the addition of "lecithin" to the diet. Two of the animals were in good condition when the experiments were terminated. The livers of these two animals were normal in appearance and contained 2.7 and 3.7 per cent fat. The iodine numbers of the fat were 118 and 152. In the second of these experiments cod liver oil had been substituted for the "lecithin." It is apparent from these results that the pancreatic enzymes are not essential to the life of the animal. In the experiments of Penau and Simonnet which are mentioned above, the diet contained large amounts of milk and bread.

It will be noted that with the onset of the typical condition the sugar excretion of these animals becomes markedly reduced and it is often necessary to decrease the dose of insulin to avoid dangerous levels of hypoglycemia. This is another illustration of the apparent alleviation of the diabetic condition of completely depancreatized animals under special conditions. It is difficult to conceive that this apparent improvement indicates a resumption of the ability of the animal to oxidize dextrose. This matter has been discussed in detail in connection with other experiments (Soskin, 1930). In numerous instances it has been demonstrated that the sugar excretion of these animals or their tolerance for insulin or both have been very considerably increased by the "lecithin." The urinary volume increases. We believe that the explanation of this result is that the liver has regained its power of forming sugar. The appearance of these increased amounts of sugar in the urine and the disappearance of fat from the liver suggest that useful data on the vexed question of gluconeogenesis from fat may be obtained by further studies in these animals.

The effects of "lecithin" on the sugar excretion of depancreatized dogs kept under conditions which were different from those existing in our experiments has been studied by one of us (S. S., 1929).

In addition to the condition which has already been discussed, there appeared in certain of the dogs symptoms which were attributed to vitamin deficiencies. The condition of the first dog placed on the experiment became very severe. Within two or three days after the diet was supplemented with food rich in vitamins, a very definite improvement was observed. To our surprise the animal died during the night about a week after the change in diet. To all appearances convulsions had preceded death, probably due to an inability of the animal to tolerate the insulin which had been given.

In addition to the first animal, dogs 2 and 3 developed convulsions, thought to be of dietary origin. Dog 3 apparently recovered spontaneously, while dog 2 failed to recover. The blood calcium of dog 3 was found to be normal during the convulsions and after recovery. Hypoglycemia was not a contributing factor in either of these cases. In view of the negative results of blood analysis, it seems probable that the convulsions were due to vitamin B deficiency. Diets deficient in this regard are well known to favor the development of convulsions in dogs (Karr, 1920; Cowgill, 1921). Other symptoms pointing to a deficiency of vitamin B in the diet were the passage of blood in the stools and diarrhea (McCollum and Simonds, 1927), and the stiffness of the limbs suggestive of polyneuritis in dogs 1 and 3, and the aphonia which was particularly marked in dog 3 but also observed in dogs 2 and 4. Many attempts were made to produce barking in these dogs but with no success. It was not until the diet had been supplemented with a satisfactory source of vitamin B (brewer's yeast and later "marmite") that dogs 3 and 4 were able to bark. At first this was a very feeble squeak and the return of the normal sound was very slow. The severe ophthalmia observed in dog 3 was undoubtedly due to a vitamin A deficiency and cleared up quickly following the addition to the diet of cod liver oil.

In spite of the appearance of symptoms of deficiency diseases in a number of our animals, we feel justified in considering them as of secondary importance only. In only one case reported by Allan, Bowie, Macleod and Robinson, was there any suggestion of a condition resembling that which we have attributed to vitamin deficiency. There appears to be very little or no connection between the signs of the deficiency diseases which Hédon reported and the acute condition which he encountered following the withdrawal of pancreas from the diet. Additional support for this view is given by the results of the work of Cowgill and Mendel (1921), who found no direct relation between vitamin B and the secretory function of the pancreas, liver and salivary glands. We have found no fatty infiltration

of the liver in rats dying of a vitamin A deficiency. On the contrary the livers of these animals contained less than the normal amount of fat. Dogs 2, 3 and 4 showed signs of acute liver condition at a time when no dietary deficiency symptoms were present. These latter symptoms appeared in spite of the frequent and in some cases almost constant administration of "lecithin," which therefore had no protective or preventive value in this regard. Purified lipins have not been found to contain any of the vitamins (MacLean and MacLean, 1927). The results of biological assays carried out by Mr. Hugh Branion of the Department of Biochemistry and Dr. E. W. McHenry of the Connaught Laboratories show that the "lecithin" used in our experiments contained no vitamin A or C. The antineuritic vitamin was present in small amounts, but apparently not in sufficient quantity to protect our animals. A more complete report dealing with the question of vitamins will be made at a later time.

It should be emphasized that the "lecithin" which we have used was prepared from egg yolk by the customary procedure of defatting with gasolene and extraction of the dried material with alcohol. In addition to lecithin, cephalin and various impurities are undoubtedly present. Experiments in which various components of the "lecithin" are being used and in which complete metabolic studies are being made, are in progress and the results will be reported in due course. Considerable time might have been saved in this work if large quantities of pure lecithin had been available.

SUMMARY AND CONCLUSION

The results of our experiments establish the fact that depancreatized dogs receiving insulin and a cane sugar and lean beef muscle diet will survive for a very long period, presumably "indefinitely," without pancreatic enzymes being available, provided that they receive frequent doses of egg yolk "lecithin." When depancreatized dogs do develop the characteristic syndrome which we attribute largely to failure of liver function the addition of "lecithin" to their diet regularly and promptly alleviates the signs and symptoms of this condition. These beneficial effects of "lecithin" suggest that the liver function is improved and this suggestion is supported by the results of the autopsy findings. Evidence is presented which makes it very unlikely that vitamins play any rôle in the development or alleviation of this condition. The direction which further work will follow is indicated.

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STUDIES ON THYROGLOBULIN

I. The Digestibility of Thyroglobulin

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Thyroglobulin, the peculiar iodine-containing protein of the thyroid gland, was first prepared by Oswald (1). Numerous investigations on this compound have appeared but a complete study has never been made. Interest in this protein was revived when Hektoen, Carlson and Schulhof (2) demonstrated it in the lymph coming from the thyroid gland. These same investigators (3) later detected thyroglobulin in the thyroid venous blood. Hicks (4) confirmed the presence of this protein in both fluids.

Since these investigations indicate one possible iodine compound entering the blood from the thyroid gland, a detailed investigation of thyroglobulin was started. There is reason to believe that a pure thyroglobulin has never been prepared since the methods employed are the same as for most globulins. Variation in iodine content of different preparations is also indicative of impurity. In attempting to separate the iodine-containing protein from others probably present, interest in the present problem arose. Hektoen, Kanai and Dragstedt (5) observed that when dogs were fed beef thyroids, beef thyroglobulin could be detected in the dog's blood, using the precipitin method. It seems illogical to assume that amino acids from beef thyroglobulin would be reconverted into beef thyroglobulin after absorption in the dog. It would appear more reasonable that some of the protein had been absorbed unchanged. The present problem has therefore a twofold interest: 1, it might be possible to separate other proteins from the iodine-containing one by a resistance of the latter to digestion, and 2, when thyroid preparations are fed, does the most of it go through the intestinal wall as thyroglobulin?

Methods. To test the first point, thyroglobulin, prepared by Oswald's method, was placed in a collodion bag with a solution of hydrochloric acid containing pepsin. The bag was surrounded with the same acid to prevent dilution by osmosis. It was hoped that other globulins (possibly present) might be digested at a more rapid rate than the iodine-containing compound. The digested products would then dialyze through the collodion leaving the iodine complex behind. It was found, however, that

iodine soon appeared on the outside of the membrane indicating digestion of the iodine-containing protein. The iodine concentration on the outside of the membrane steadily increased until at the end of 36 hours, 33 per cent of the total iodine had diffused to the outside. Much of the fluid outside had gone into the bag so that it is safe to say that a considerable portion of the total iodine was in a diffusible state. The results of four experiments were similar. A control experiment showed less than one-fourth as much diffusion when pepsin was not added to the solution in the bag. The ratio of nitrogen to iodine inside and outside the bag showed that no concentration of iodine was being effected. No inorganic iodine was detected by the nitrite test. This procedure for the purification of thyroglobulin was abandoned but the action of enzymes on the protein was continued in order to throw light on the second point above.

Other investigators have reported that thyroglobulin was digestible but only qualitative experiments were carried out. Thus Nurnberg (6) reported that trypsin set free inorganic iodine. Examination of his data reveals that the mixtures were left standing for several months before analysis. It is difficult to say how much his results resemble the normal action or whether bacterial decomposition may have played a part. At any rate it is evident that his results are not comparable with experiments of shorter duration or what might happen in the living animal. In view of the fact that thyroid medication is so efficacious orally, it seems unlikely that much inorganic iodine would be split off during normal digestion.

After the work was begun, a paper by Harrington and Salter (7) appeared in which they state that they found very little inorganic iodine liberated by enzymes over short periods of time. These investigators report that the digestive enzymes attack the iodine-containing protein in vitro. The present work confirms this point.

The action of pepsin and trypsin has been studied by two methods: 1, the Sorensen formol titration has been applied to the digestive mixture, and 2, precipitation reactions with ammonium sulfate have been employed to determine the nature of the iodine complex. Finally the latter method has been applied to the contents of the gut and to the blood during normal digestion of thyroglobulin.

Formol titration. This method, although indicative, does not yield crucial information here. It gives only the increase in carboxyl groups without telling anything about what has happened to the iodine. If other globulins are present, the increased titration might be due to their digestive products. A few experiments were carried out in the usual manner of bringing the sample to the neutral point, adding neutralized formalin and titrating with 0.1 N NaOH again to neutrality. Large concentrations of enzyme were employed (one gram to 15 grams of protein). The protein was mixed with pepsin and hydrochloric acid. Digestion was allowed

to proceed for two or three days when the mixture was made approximately neutral to litmus. Sodium bicarbonate powder was added to a concentration of one per cent. Pancreatin was then added and digestion continued. At definite intervals samples were withdrawn and analyzed. The following curve gives the results of three such experiments. The work was not continued and compared with other proteins for the reason given above. At point *A* trypsin was added to the first sample and at point *B* it was added to the other two.

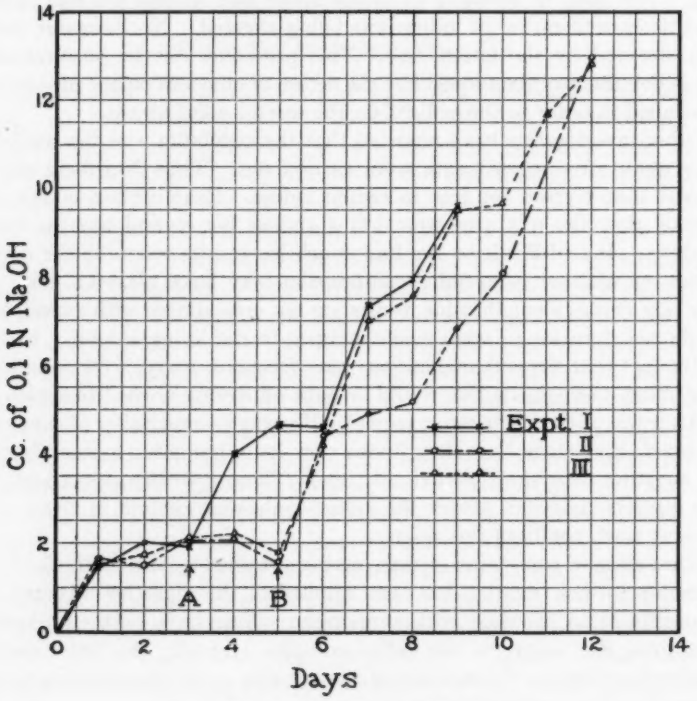

Fig. 1. Formol titration

Precipitation with ammonium sulfate. This method is much better for the problem at hand. Iodine estimations were made on the digestion mixtures. A sample was withdrawn and mixed with an equal volume of ammonium sulfate. The primary albumoses were filtered off and an iodine estimation made on the filtrate. The remainder of the filtrate was saturated with ammonium sulfate and the precipitate again filtered off. An iodine estimation on the saturated filtrate gives the quantity of iodine present in peptone combination or smaller digestive products. Using this

method it was found that in 36 hours, pepsin had appreciably changed thyroglobulin. After one-half saturation with ammonium sulfate, 30.6 per cent of the iodine was left in solution and 13.6 per cent after complete saturation. Both of these values increased with time. At the end of 84 hours only 62.5 per cent could be removed by completely saturating the solution.

The action of trypsin was much more rapid. One really should follow the action of trypsin after peptic digestion but in the present study, the trypsin was added directly to the original protein. Striking results were obtained even on the raw protein. It was found that an appreciable quantity of iodine was not precipitated at full saturation with ammonium sulfate when the trypsin had acted for three hours. At the end of 25 hours, no precipitate formed at half saturation and only 35 per cent of the iodine was removed by completely saturating with ammonium sulfate. Five experiments were carried out by the precipitation method. These experiments indicate that thyroglobulin is readily attacked by both pepsin and trypsin. Although it was not considered worth while to compare the rate of digestion with other proteins, one might predict that under normal conditions in the living animal, this protein would not be an exception to the action of digestive juices.

Work on dogs. Having definitely established that thyroglobulin was readily acted upon by digestive enzymes, a few experiments were carried out to see what would happen in the living animal under normal conditions. Dogs were fasted 48 hours. They were then fed their normal diet of bread, meat, and bone meal to which were added 50 grams of thyroglobulin. The first animal was also fed some $BaSO_4$ in order to observe the progress of the meal but this led to complications such as adsorption and interference with the analysis. At the end of six hours, the animals were given barbital, a quantity of blood was withdrawn and the animal finally killed with chloroform. The gut was quickly removed and divided into three segments. The contents of each were washed out and analyzed separately.

Stomach. The stomach contents were mixed with about 200 cc. of 0.9 per cent saline. The acidity of the contents made the mixture definitely acid. The solution was allowed to extract for 24 hours, then decanted and more saline added together with enough NaOH to make the reaction definitely alkaline. It will be remembered that thyroglobulin is insoluble in acid but readily soluble in alkali. The alkaline solution was filtered after setting in the ice box for 24 hours. Both of these extractions did not remove all of the iodine from the stomach contents since unquestionably some would be adsorbed on other proteins. Further extraction would remove more but the first two extracts are probably representative of the total. Furthermore, the stomach digestion is not so important as the

intestinal since it is possible that some thyroglobulin might enter the intestine without having been acted upon by pepsin.

Intestines. The small intestine was divided into two segments and the contents extracted with 0.9 per cent saline which was made definitely alkaline. This removed practically all of the iodine. The lower segment is termed colon for convenience but it contained considerable material from the lower intestine. After extracting 24 hours, these mixtures were centrifuged and the supernatant fluid analyzed as mentioned above.

TABLE 1

Showing the total iodine in each segment

DOG	ACID STOMACH	BASIC STOMACH	INTESTINE	COLON
	mgm.	*mgm.*	*mgm.*	*mgm.*
1	13.8	46.6	8.1	13.6
2	7.0	54.0	7.0	7.5
3	4.9	7.0	12.7	59.0
4	3.3	22.4	20.5	26.4

TABLE 2

Showing the percentage of iodine removed by half saturation

DOG	ACID STOMACH	BASIC STOMACH	INTESTINE	COLON
1	0	25.0	0	0
2	0	92.0	0	61.3
3	10.2	21.4	29.1	89.0
4	21.4	80.0	30.0	79.2

TABLE 3

Showing the percentage of iodine removed by complete saturation

DOG	ACID STOMACH	BASIC STOMACH	INTESTINE	COLON
1	Little	Much	14.7	70.0
2	10.0	93.3	42.9	85.3
3	22.2	35.0	72.0	95.0
4	21.4	86.7	79.5	92.0

Table 1 shows the total quantity of iodine in each extract and tables 2 and 3 show the percentage of that iodine which is removed by one-half and complete saturation respectively. The analyses were somewhat doubtful after full saturation in the first dog's stomach extract. The $BaSO_4$ may have been partly responsible. For that reason, the amount of precipitate was estimated and not reported in percentage.

Discussion. Studies of this nature will not check very closely due to

the marked variation in the rate of digestion in different animals. The first two dogs were small and much food was left in the stomach. The last two animals were unusually large and digestion in the stomach was nearer completion. This is especially true of dog 3, where it can be seen that only 7 mgm. of iodine remained in the stomach while 59 mgm. were in the colon. In spite of the variation in results, it seems obvious that the thyroglobulin is digested.

In table 2 it can be seen that the iodine in the acid stomach extract is in the form of small molecules. The highest percentage removed by half saturation is 21.4.. The basic stomach extracts vary somewhat. This would be expected since this fraction may contain some of the original protein if the contents of the stomach had not yet come into contact with the pepsin. The intestinal segments are the most significant. The highest quantity removed by half saturation was 30.0 per cent. Thus, over two-thirds of the iodine present is far removed from thyroglobulin and it seems unlikely that much of he remaining 30 per cent would be in the original form. It must be remembered that a higher concentration of small molecules could also result from a more rapid absorption of large molecules. Assuming for the moment that this is true, what would be expected in the colon segment? There should be an accumulation of the remaining small molecules in this region. The last column of table 2 shows that most of the iodine in the colon extract is removed by half saturation. In other words, the larger molecules are not absorbed but pass on down the gut. From these facts, it seems logical to assume that the most of the iodine entering the blood stream is not in the form of thyroglobulin.

Table 3 serves more as a check on the previous table. It is interesting that no more of the iodine in the acid stomach extracts is taken out by complete saturation. The low percentage for dog 3 in the basic stomach extract is due to the fact that peptic digestion was practically complete. The variation in the intestinal extracts cannot be explained, but the rate of emptying time for the stomach would certainly be a factor. The colon results are consistent and what one would expect if some of the protein escapes digestion and is not absorbed.

If thyroglobulin is digested similar to most proteins as the above data indicate, one might postulate that the iodine in the blood stream would be combined with small molecules. To test this assumption the blood of the last three dogs was analyzed for iodine before and after half saturation with ammonium sulfate. The iodine content of the blood was between 0.6 and 1.0 mgm. per 100 cc. of blood. None of this was removed by half saturation in any of the three samples. This would seem conclusive proof that thyroglobulin had not been absorbed undigested but the possibility remains that thyroglobulin might be digested after entering the blood. No doubt it would be broken down if it got into the blood, but from the

intestinal analyses it seems unlikely that a significant quantity escapes digestion. Therefore the state of iodine in the blood may be taken as confirmatory evidence that digestion has occurred.

This quantitative work does not detract from the interesting observation of Hektoen, Kanai and Dragstedt. It is interesting if some of the original protein can pass into the blood since there is embryological evidence that the thyroid gland once poured its secretion into the alimentary canal.

SUMMARY

1. Quantitative studies are reported which show that thyroglobulin is easily digested in vitro by pepsin and trypsin.

2. The Sorensen formol titration shows a steady increase in the carboxyl groups.

3. Precipitation reactions with ammonium sulfate show that the iodine containing protein is being digested.

4. Analysis of the gastro-intestinal tract during normal digestion indicates that thyroglobulin is digested in a normal manner.

5. None of the iodine in the blood during digestion of thyroglobulin is precipitated by half-saturated ammonium sulfate.

We wish to thank Doctor Fenger of Armour & Company who kindly furnished us with fresh thyroids.

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CUTANEOUS RESPIRATION IN MAN

III. The Permeability of the Skin to Carbon Dioxide and Oxygen as Affected by Altering their Tension in the Air Surrounding the Skin

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Shaw, Messer and Weiss (1929) and Shaw and Messer (1930) have shown that under normal conditions the skin excretes carbon dioxide into and absorbs oxygen from the air, the rate depending chiefly upon the temperature and relative humidity of the air. Since the skin may receive its oxygen from the blood as well as from the air, and since the carbon dioxide may be excreted into either the blood or the air, we have undertaken the present research to determine the relative dependence of the skin upon the air and the blood in performing its respiratory functions.

Method. The subject lay on a couch with the entire arm in a plethysmograph. The apparatus and technical procedure were essentially the same as those employed by Shaw, Messer and Weiss (1929), with additional precautions to prevent the possibility of leakage. The experiment lasted for three hours. Gas samples were withdrawn from the plethysmograph, and the change in percentage composition of the carbon dioxide and oxygen multiplied by the total volume of gas in contact with the skin gave the volume of carbon dioxide and oxygen which had been gained or lost during a given period.

The experiments were done under conditions which were uniform with respect to the principal factors affecting the rate of cutaneous respiration. It has been shown by Shaw and Messer (1930) that this rate is affected both by the temperature and by the relative humidity of the air in contact with the skin. Shaw, Messer and Weiss (1929) have shown that the rate is also affected by individual characteristics and by seasonal changes. Except in one series of experiments, all the observations were made upon the same subject, with the air in contact with the skin at a constant temperature (31.0° ± 0.5°C.) and at a constant relative humidity at the saturation point. The seasonal effect could not be altogether eliminated, owing to the fact that the experiments were done over a period extending from April 22 to July 24. It will be shown, however, that only those

experiments done between April 22 and May 26 were affected by change of season, those subsequent to May 26 indicating a steady state.

When the plethysmograph is filled with room air, a slight inward leak causes no appreciable alteration in the concentration of the carbon dioxide and oxygen in contact with the skin; but when the plethysmograph is filled with a gas having a composition different from air, a very slight inward leak would invalidate the results. In order to test the efficacy of our apparatus control experiments were carried out in which the plethys-

TABLE 1

Rate of carbon dioxide and oxygen diffusion through skin as affected by altering the carbon dioxide concentration in the air around the skin with the oxygen concentration held constant at about 20 per cent (subject A. F. S.)

NUMBER OF EXPERIMENT	CARBON DIOXIDE IN AIR AROUND SKIN	(A) CARBON DIOXIDE*		(B) OXYGEN*	
		+ Excreted − Absorbed	Average	Absorbed	Average
	per cent	*cc.*		*cc.*	*cc.*
46	0.5	+129	0.5 per cent	84	
47	0.5	+132		97	94.3
48	0.5	+131	+131 cc.	102	
26	4.10	+81	4.34 per cent	82	
27	4.40	+86		78	82
20	4.55	+78	+82 cc.	88	
44	8.75	+12		62	
53	9.29	+13		74	
54	9.50	+8	9.56 per cent	67	64
55	9.84	−5		63	
35	9.91	−34	−1 cc.	53	
34	10.10	−3		65	
39	14.45	−113	14.91 per cent	30	
40	15.04	−95		25	24
43	15.25	−124	−111 cc.	16	

* Calculated in cubic centimeters per hour per square meter of skin surface.

mograph was filled with gas mixtures differing in composition from room air, and the aperture through which the arm passed was plugged with a bottle. The analyses for carbon dioxide and oxygen at 1-hour intervals were as follows: experiment 1, per cent carbon dioxide 6.28, 6.29 and 6.27, and per cent oxygen 11.89, 11.90 and 11.91; experiment 2, per cent carbon dioxide 7.86, 7.86, 7.87 and 7.86, and per cent oxygen 7.74, 7.77, 7.74 and 7.74. In these experiments the concentration of carbon dioxide and oxygen held constant within the analytical error, which for the former is

±0.01 per cent, and for the latter is ±0.02 per cent. The error in calculating the changes in the volume of carbon dioxide and oxygen of the gas in contact with the skin under the conditions of our experiments was ±1.3 cc. for carbon dioxide and ±1.7 cc. for oxygen.

EXPERIMENTAL RESULTS. *The effect upon cutaneous respiration of increasing the carbon dioxide concentration in the air.* The skin was surrounded by air having a carbon dioxide concentration which varied from 0 to 15 per cent, with a constant oxygen concentration of 20 per cent, and the balance nitrogen. Under these conditions carbon dioxide was excreted

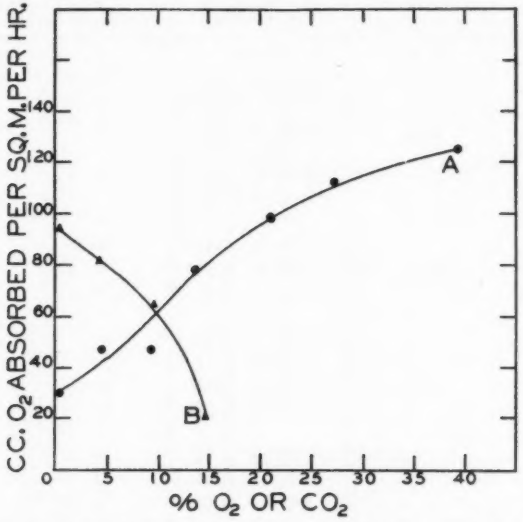

Fig. 1. Curve *A*, rate of oxygen absorption, the oxygen concentration in the air around the skin rising from 0 to 40 per cent and the carbon dioxide held constant at about 0.5 per cent. Curve *B*, rate of oxygen absorption, the carbon dioxide concentration of the air around the skin rising from 0 to 15 per cent and the oxygen held constant at 20 per cent.

by the skin at a rate which diminished progressively as the concentration of the carbon dioxide in the air increased up to 9.50 per cent. At concentrations exceeding 9.50 per cent, carbon dioxide was absorbed (table 1, column A). Oxygen was absorbed, but at a rate which diminished progressively as the concentration of the carbon dioxide increased (table 1, column B; and fig. 1, curve *B*).

The carbon dioxide equilibrium between the air and the skin. From the above observations upon the permeability of carbon dioxide through the skin, it was concluded that if the tension could be found at which the

carbon dioxide in the plethysmograph was neither diminished nor increased, that tension would be equal to and representative of the normal carbon dioxide tension in the skin. With this object in view a series of observations was made upon five different subjects. The data are given in table 2. When oxygen was present at the normal concentration, carbon

TABLE 2

The carbon dioxide equilibrium between the air and the skin as indicated by the transition from excretion to absorption

SUBJECT	OXYGEN PRESENT	PER CENT CHANGE IN CARBON DIOXIDE CONCENTRATION	+ EXCRETED − ABSORBED	EQUILIBRIUM CARBON DIOXIDE TENSION
	per cent	*per cent*		*mm.* Hg
A. F. S.	20.0	8.72 to 8.81	+	74
		9.24 to 9.34	+	
		9.46 to 9.53	+	
		9.87 to 9.82	−	
		10.05 to 9.78	−	
		10.12 to 10.09	−	
A. E. T.	20.0	8.35 to 8.43	+	71
		8.69 to 8.78	+	
		9.37 to 9.35	−	
	0.5	7.63 to 7.84	+	61
		8.10 to 8.11	+	
		8.71 to 8.61	−	
		9.08 to 9.03		
R. H.	0.5	7.43 to 7.72	+	66
		8.21 to 8.44	+	
		9.08 to 9.02	−	
M. H. L.	0.5	9.44 to 9.31	−	72
		9.47 to 9.48	+	
		9.49 to 9.49	0	
M. W. A.	0.5	8.22 to 8.27	+	71
		8.62 to 8.66	+	
		8.90 to 8.94	+	
		9.81 to 9.75	−	

dioxide was neither excreted nor absorbed at tensions of 74 and 71 mm. of mercury. When the oxygen concentration was reduced to 0.5 per cent, the carbon dioxide tensions at equilibrium were 61, 66, 72 and 71 mm. of mercury.

In the experiments on A. F. S. and the first three on A. E. T., the carbon dioxide tension in the skin seemed so high as to cause the suspicion that

superficial oxidation was taking place and that carbon dioxide was passing into the air more rapidly than it could be absorbed by the skin, thereby establishing equilibrium at an abnormally high level. The reduction of the oxygen concentration to a fraction of 1 per cent in all the subsequent

TABLE 3

Rate of carbon dioxide and oxygen diffusion through skin as affected by altering the oxygen concentration in the air around the skin with the carbon dioxide concentration held constant at about 0.5 per cent (subject A. F. S.)

NUMBER OF EXPERIMENT	OXYGEN IN AIR AROUND SKIN	(A) OXYGEN*		(B) CARBON DIOXIDE* EXCRETED	DATE
		Absorbed	Average		
	per cent	*cc.*		*cc.*	
13	0.84	6	0.76 per cent	182	April 22
14	0.69	2	4 cc.	171	April 23
56	4.80	48	4.52 per cent	133	July 22
57	4.24	46		132	July 23
58	4.51	47	47 cc.	122	July 24
24	9.46	57	9.29 per cent	138	May 12
25	9.12	48	52 cc.	135	May 13
49	13.9	74	13.75 per cent	127	July 7
50	13.8	83	78 cc.	133	July 8
15	20.75	96		159	April 25
16	20.75	103		152	April 29
17	20.75	107	20.75 per cent	152	April 30
22	20.75	92		152	May 8
23	20.75	104		149	May 9
46	20.75	84	98 cc.	129	June 25
47	20.75	97		132	June 26
48	20.75	102		131	June 27
51	27.26	112	27.25 per cent	128	July 9
52	27.25	113	112 cc.	130	July 10
32	40.80	131	39.44 per cent	129	May 26
33	38.08	119	125 cc.	128	May 27

* Calculated in cubic centimeters per hour per square meter of skin surface.

experiments was made in order to eliminate such a possibility. It is apparent from the experiments on A. E. T., who was first tested with 20 per cent oxygen and then with 0.5 per cent oxygen, that this suspicion was to some measure justified, the carbon dioxide tension of equilibrium falling from 71 to 61 mm. as a result of excluding the oxygen. As a

consequence the experiments on R. H., M. H. L. and M. W. A. were done at oxygen concentrations of 0.5 per cent. These still show carbon dioxide equilibrium tensions in the skin which are very high, and it is of interest that even at the low oxygen tension, oxygen was still being absorbed. The disappearance of oxygen at this low tension can only be explained by superficial oxidation, unless one supposes that oxygen can pass into the tissues against a tension far higher than that of the surrounding air.

So long as superficial oxidation with the consequent production of carbon dioxide is taking place, one must be cautious in accepting the carbon dioxide equilibrium between air and skin as the carbon dioxide tension in the skin.

The effect upon cutaneous respiration of altering the oxygen concentration in the air. The skin was surrounded by gas mixtures having an oxygen concentration which varied from 0.69 to 40 per cent, with no carbon dioxide, and the balance nitrogen. Oxygen was absorbed under all conditions, the rate of absorption increasing as the oxygen per cent of the air increased (table 3, column A). The rate of carbon dioxide excretion was not influenced by altering the oxygen concentration (table 3, column B), the variations which do exist being referable to a seasonal change. When the rate of carbon dioxide excretion is tabulated in order of the date of the experiments, it is found that the rate falls from 182 cc. on April 22 to 129 cc. on May 26 in a perfectly steady manner without a single case of regression, and subsequent to May 26 the rate remains very constant at about 130 cc. The conclusion is thus justified that the oxygen concentration has no effect upon the rate of carbon dioxide diffusion through the skin.

The rate of oxygen absorption with increasing oxygen concentration is plotted in graphic form in figure 1, curve *A*. The first point in the curve was taken from an average of 14 experiments on 4 subjects, in which the initial oxygen concentration was 0.5 per cent falling to 0.3 per cent with an oxygen absorption of 15 to 41 cc. (average 25 cc.); the remaining points were constructed from the data derived from subject A. F. S. shown in table 3.

Discussion. Shaw, Messer and Weiss (1929) showed that there was a 46 per cent increase in the rate of carbon dioxide excretion with the onset of cold weather. Their findings were confirmed by our experiments (table 3, column B) in which we found a 30 per cent diminution in the rate of carbon dioxide excretion with the onset of warm weather.

Campbell (1929), using a plethysmographic method very similar to ours, found that equilibrium between the carbon dioxide in the skin and the air was established at a tension of about 40 mm. of mercury. This figure seems more reasonable than our own figures which are much

higher. This discrepancy is unexplainable unless he took the precaution to exclude even a trace of oxygen from the carbon dioxide gas mixtures surrounding the arm, thereby eliminating the production of carbon dioxide in the plethysmograph by superficial oxidation. Campbell also found that the skin was impermeable to the outward passage of oxygen, but failed to observe the passage inward until the tension of oxygen in the air had reached 50 mm.

The fact that oxygen continues to be absorbed by the skin even when the oxygen tension of the surrounding air is as low as 3 or 4 mm. is contradictory to a statement made by Shaw, Messer and Weiss (1929) to the effect that "when the oxygen of the air in contact with the skin was reduced to about 2 per cent, then oxygen was given off by the skin." In the light of more extensive experimentation with more highly perfected apparatus, we are now convinced that the increased oxygen content of the plethysmograph was due to a slight leak or diffusion inward of the room air.

If it is assumed that the oxygen tension in the skin is at least 40 mm., as is indicated by the work of Campbell (1926) and of Bazett and Sribyatta (1928) who determined oxygen tensions in the subcutaneous tissue, then the oxygen absorbed from the air at tensions lower than 40 mm. must certainly indicate superficial oxidation rather than diffusion inward against a higher oxygen pressure. When it is considered that at an oxygen tension of 3 or 4 mm. about 25 per cent as much oxygen is absorbed from the air as at an oxygen tension of 157 mm. (normal air), one is impressed by the avidity which the skin evinces toward oxygen at low tensions. It is quite possible that all of the oxygen passing into the skin is utilized in tissue oxidation, and that the higher the oxygen content of the air the greater is the utilization of oxygen from external sources rather than from the blood.

The fall in the rate of oxygen absorption with a rise in the carbon dioxide concentration could not be caused by an inhibition of oxidation due to the toxic effect of carbon dioxide, since we have on several occasions made intact cats breathe 15 per cent carbon dioxide in air for a sufficient period of time to bring most of the tissues of the body into equilibrium with the alveolar air, without the slightest effect upon the well-being of the animal. There can be little doubt, however, that by increasing the carbon dioxide concentration of the air, the oxygen tension in the blood is raised in the following manner: When the carbon dioxide tension of the air is raised above that of the blood, the carbon dioxide forming in the skin will be forced into the blood rather than escape into the air, and there will be added to this the carbon dioxide passing through the skin from the air. Under these conditions the carbon dioxide tension in the blood will be greatly increased, and at the same time the oxygen tension of the blood will be increased by the shifting of the oxygen dissociation curve to the

right. It is conceivable that such a shift in the oxygen dissociation curve might raise the oxygen tension of the blood from 40 mm. up to 60 mm. and thereby reduce somewhat the oxygen tension difference between the skin and the air, and cause a reduction of the diffusion pressure. But even at this increased tension, diffusion of oxygen from the air into the skin should continue at a rate which is only slightly below normal. It is far more probable that as the oxygen tension in the blood is raised above the normal level the metabolic requirements of the skin are met more completely by oxygen from the blood and, as a result, the demand for oxygen from the air is proportionately reduced.

Our experiments show definitely that the skin is impermeable to the outward passage of oxygen. They indicate that the oxygen that passes inward is utilized in the metabolism of the skin, and that the rate at which oxygen is absorbed from the air will depend upon the relative tensions of oxygen in the blood and in the air. With a constant oxygen tension of the blood, the rate of oxygen absorption from air will vary directly with the oxygen tension of the air; and with a constant oxygen tension of the air, the rate of absorption will vary inversely with the oxygen tension of the blood. The great irregularity of the respiratory quotient (Shaw and Messer, 1930; and Shaw, Messer and Weiss, 1929) under normal conditions is undoubtedly a reflection of the fact that with every change in the oxygen tension of the blood there is a reciprocal change in the demand of the skin for oxygen of the air.

Since the carbon dioxide that is formed in the skin may escape into the blood as well as into the air, and since the oxygen utilized may be drawn from both blood and air, neither the rate of carbon dioxide excretion nor of oxygen absorption will constitute an absolute measure of the rate of metabolism in the skin, but it is undoubtedly a fairly reliable index of the relative rates of metabolism.

SUMMARY

1. When the skin was surrounded by air containing approximately 8.5 per cent carbon dioxide, a condition of equilibrium was established. When the concentration was diminished, carbon dioxide passed from the skin into the air; and when the concentration was increased, then carbon dioxide passed from the air into the skin.

2. Oxygen was absorbed by the skin even when the air contained less than 0.5 per cent. Under no conditions was the passage of oxygen from the skin into the air observed.

3. The avidity of the skin for the oxygen of the air at tensions as low as 3 or 4 mm. of mercury affords evidence that the skin, under normal conditions, must utilize the oxygen available from this source in addition to that supplied by the blood.

4. The rate of oxygen absorption decreases progressively as the carbon dioxide tension of the air increases.

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THE EXCITABILITY OF THE TURTLE VENTRICLE DURING VAGUS STIMULATION

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In every species of vertebrate hitherto tested, both stimulation of the vagus (1), (2) and the application of drugs which have a vagus-like action (pilocarpine, eserin, acetyl choline) (3), (4) have been reported to shorten the chronaxie of the ventricle. This effect of the vagus is stated to be independent of an increase in the rheobase, which is said not to change regularly in either direction. The tendency to explain the reported shortened chronaxie as an indirect result of vagus slowing has been controverted, since in the experiments of Field and Brücke (5) (frog) and of Fredericq (6) (turtle) the ventricular rate was kept constant by rhythmic electrical stimulation, and, in Fredericq and Garrey's (7) experiments, advantage was taken of the fact that frequently strong stimulation of the left vagus did not change the rate of the turtle heart, although producing marked inotropic effects in the auricles, especially the left.

With the chronaxie determination on animals whose ventricles are known to receive direct vagus innervation this investigation has no immediate concern (cf. Fredericq (8) for bibliography), but the reported changes in the chronaxie of the ventricle of the turtle obviously demand renewed consideration, since the reported inhibitory effects of the vagus, and therefore the changes in chronaxie ascribed to vagus stimulation are questionable. Gaskell (9) long ago demonstrated that the beat of the turtle ventricle is not weakened by vagus stimulation, a finding which has been abundantly confirmed by others for various chelonian species. Since, in the auricle, the other properties of the muscle, the excitability, the conductivity, and possibly the tonicity, are affected by vagus stimulation, the question arises whether in the ventricle one of these properties, viz., the excitability, or that special aspect of excitability represented by the chronaxie, may be affected independently of any demonstrable change in the other properties of the cardiac muscle. Garrey (10) has reported occasional cessation of ventricular fibrillation in the turtle subsequent to vagus stimulation, but this is readily explained since the path of the circus contraction probably included junctional tissues influenced by the vagus;

thus the effect does not necessarily mean inhibition of ventricular muscle proper. The changes in chronaxie remain, then, as the only reported quantitative evidence that the vagus has any influence whatever on the ventricle of the turtle.

We have reinvestigated this question and this paper gives the results of our measurements of thresholds and chronaxies in the turtle ventricle during vagus stimulation.

METHOD. After destroying the brain and spinal cord of the turtles (9–10 inch *Chelydra serpentina* and 6–8 inch *Chrysemys sp.*) and removing the plastron, the vagi were dissected out in the neck along with the carotid arteries, and the heart was exposed. In all cases the ventricular systoles were recorded by the suspension method, together with time in seconds and signals for vagus stimulation and testing stimuli of the ventricle. The rheobase and chronaxie were then determined at appropriate intervals, and occasionally data were obtained relating intensity to minimal excitation time over a considerable range of stimulus strength. For this purpose we employed, in the prescribed manner, the Lapicque apparatus, including the potential reducer (potentiometer), a resistance in series with the preparation (generally 7000 ohms), and the chronaximeter (rheotome). The stimulating electrode was a small silver hook, supported by a spiral of fine copper wire to permit free movement, which was hooked into and through the epicardium, always near the ventricular base. When the hook was placed on one side of the base, the vagus on that side, or more usually, both vagi were stimulated. The indifferent electrode was a large silver wire, its end bent in zig-zag fashion to present a large surface to the abdominal viscera among which it was placed.

Data were obtained from the ventricles under a variety of conditions: 1, beating slowly (sinus only cooled) and driven by rhythmic induction shocks applied at the apex so that the rate was unchanged by the vagus; 2, brought to complete standstill by application of acetyl choline to the sinus of the bloodless heart; 3, brought to complete standstill by a second Stannius' ligature which did not include the coronary nerve;[1] 4, beating rhythmically excepting when slowed or stopped by vagus stimulation.

In view of the frequently observed depressing action of the constant current used for stimulation, it is important to note that in all experiments care was taken to maintain a constant time interval between the applications of the testing current, both during and without vagus stimulation.

While testing, the circuit through the potentiometer remained closed. The shunt circuit was then made and broken, for testing rheobase by means of a "mercoid" key, and for testing intensity-time relations, by the keys of the chronaximeter.

[1] This procedure is regarded as less satisfactory since no certain information was obtainable regarding the condition of the putative vago-inhibitory supply of the ventricle.

RESULTS. *The driven ventricle.* Tests were made under this condition in four hearts. In no case was there the slightest evidence, even during strong faradic stimulation of both vagi, of a change either in rheobase or chronaxie, although the tests were repeated a large number of times on every ventricle. Figure 1 illustrates the results of such an experiment. In the upper part of the figure the strengths of the threshold stimuli are

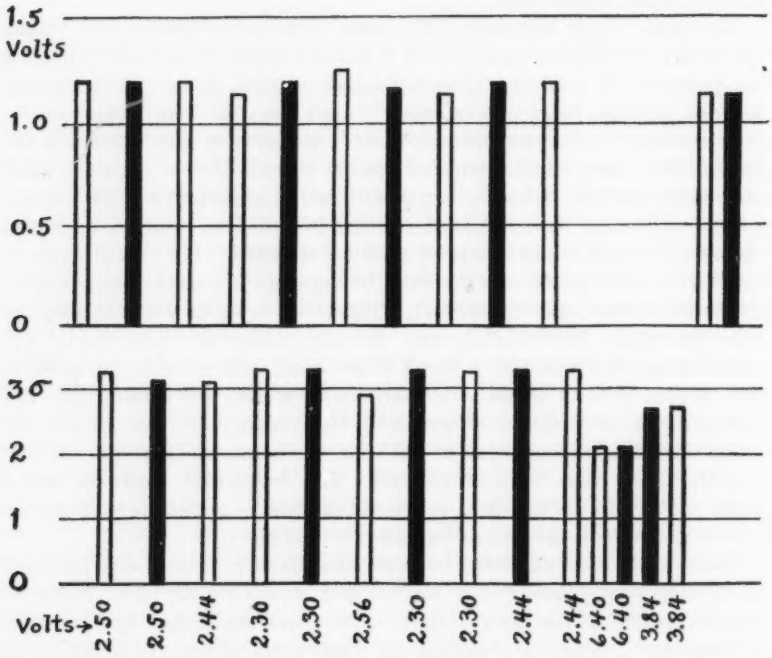

Fig. 1. Driven ventricle. *Upper line,* rheobasic determinations in chronological sequence. White columns, rheobases, vagus not stimulated; black columns, rheobases, vagus stimulated. *Lower line,* chiefly chronaxie determinations, also in chronological sequence. White columns, no vagus stimulation; black columns, vagus stimulation. The last four columns represent, not chronaxies, but least time. Below each column the current intensity actually used is indicated. Contrast this figure with figure 4 from the same ventricle but with uncontrolled rate.

shown in chronological sequence. The heights of the black columns are proportional to the rheobasic strength during vagus stimulation; and similarly the heights of the white columns are proportional to the rheobasic strength when the vagus was not stimulated. The gaps in this series of columns are the periods during which the excitation times, shown in the lower part of the figure, were being determined. The height of each of

these latter columns represents the least time necessary to elicit the response. Below each of these columns, or group of columns, is indicated the strength in volts which was used. Black and white, as before, indicate that the vagus nerve was, or was not, stimulated.

Although the figure shows that both thresholds and excitation times fluctuate somewhat, it is evident that vagus stimulation was quite without effect upon either; or, more explicitly, if there was a change it could not have been greater than about 3 per cent and have remained undetected. This result is typical for all those experiments in which the procedure was the same. In no case was there the slightest evidence of a change in excitability as tested either by rheobase or by chronaxie or by minimal excitation time in those tests in which the employed strength was not just double the rheobase.

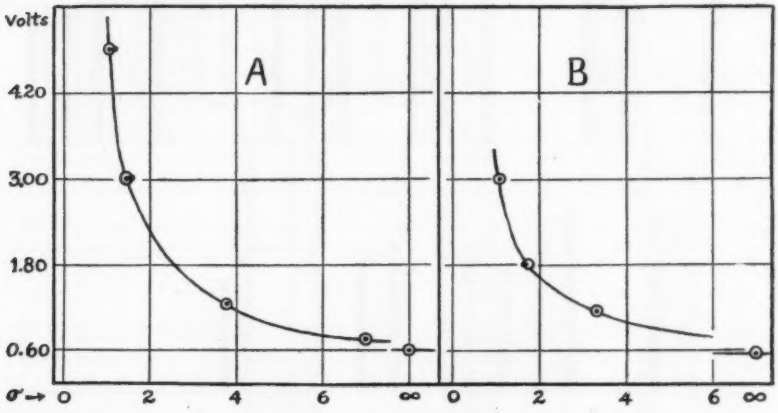

Fig. 2 A. Quiescent ventricle. Intensity-time curves; dots, during vagus stimulation, circles, without vagus stimulation. The vagus produces no effect upon least time at any intensity as is indicated by the coincidence of the curves.

Fig. 2B. Quiescent ventricle. The same as 2 A but from another heart.

The quiescent ventricle. The method here used in case of three of the four ventricles rendered quiescent, namely, the application of acetyl choline to the sinus in the heart in which the circulation had been stopped, is, perhaps, not quite so unobjectionable as the previous method. There is no absolute certainty that the drug did not diffuse in minute quantities to the ventricle and there produce some effect upon the putative vago-inhibitory endings. On the other hand, the other method of producing ventricular quiescence, i.e., that of ligating at the A-V groove with avoidance of the coronary nerve, may eliminate too much of the supposed vagus innervation of the ventricle. In either case the vagus effects may be

conceived to be reduced, but it would be gratuitous to say that they will be entirely eliminated. Yet it was found, as in case of the driven ventricle, that the quiescent or very slowly beating ventricle had undergone no detectable change in excitability, even during maximal stimulation of both vagi. The typical result is illustrated in figures 2 and 3.

The ventricle without control of rate. When the heart is beating rhythmically and is greatly slowed or stopped by vagus stimulation, quite variable results are obtained. The effects are clearly dependent upon the rate of the cardiac rhythm and upon the condition of the ventricle. Eight ventricles were tested under these conditions and of the eight only one failed to undergo a definite change in its excitability upon change in

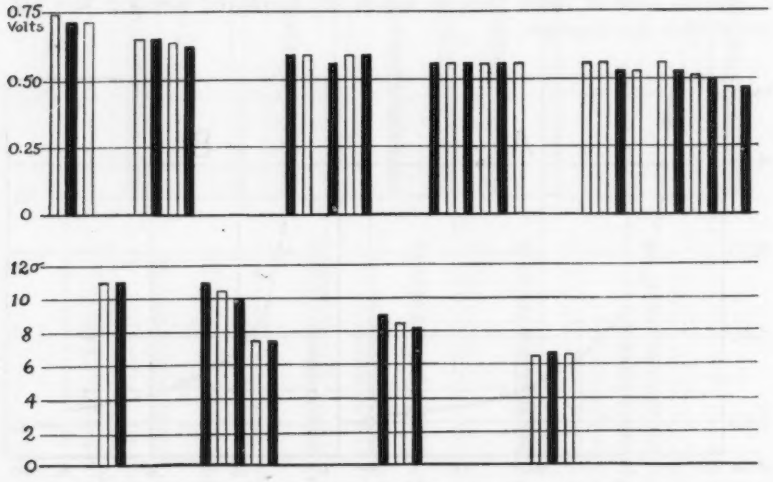

Fig. 3. Quiescent ventricle. Otherwise the same as figure 1. There was here a progressive shortening of chronaxie and, to a smaller extent, a decrease in the rheobase with lapse of time.

the rate; the exceptional heart being one in which the rhythm was so slow (one to two beats per minute), because of cooling the sinus and S-A block, that it more properly falls into the group of quiescent ventricles.

Analysis of the results on this group of hearts reveals three types of effect of vagus standstill.

1. If the ventricle is initially very slow and unfatigued, the standstill may produce no detectable change either in the rheobase or the chronaxie. This is represented by the one heart mentioned above.

2. If the initial rate is rapid, and the ventricle is somewhat fatigued, standstill will lower the rheobase and shorten the chronaxie, the former effect, so far as our data go, being more marked than the latter. This

type of effect is illustrated by two ventricles, and also probably by another in which, however, the chronaxie was not determined. In this latter example the rheobase, measured as late as possible in the diastole of the normally beating heart, was 1.45 volts. During vagus standstill the threshold fell progressively until it was 0.8 volt. It might be assumed that this was a direct effect of vagus action on the ventricular musculature, but that in reality it was due to slowing could easily be shown, for when

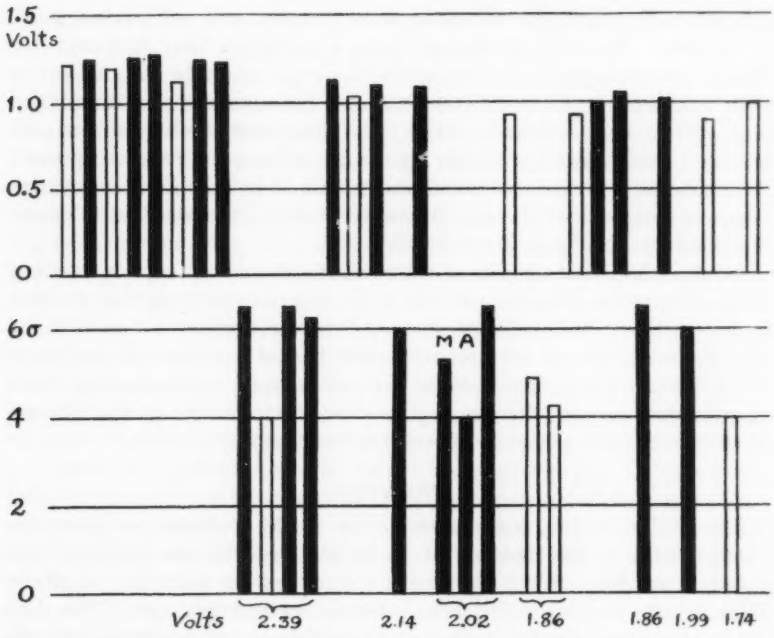

Fig. 4. Ventricle, rate uncontrolled. With vagus slowing the rheobase rose 7 or 8 per cent; the chronaxie, or here more correctly the least time, rose over 50 per cent. At *M* the ventricle was driven at a rate much slower than the sinus rhythm; at *A* the rate of driving approximated the sinus rhythm. Note that the latter rate reduced the chronaxie to the same level as that during normal rhythm.

acetyl choline was applied to the sinus with care to prevent its reaching the ventricle, thus producing quiescence of the ventricle, vagus stimulation effected no change either in the threshold or in the excitation time. It was most obvious that the whole effect had been due to change in rate and not directly to intrinsic nerve influence.

3. If the ventricle is beating initially at a moderate or even rapid rate, but is unfatigued, vagus standstill brings about a lengthening of the chron-

axie although the rheobase may show an increase, a slight decrease, or may remain unchanged. In one of the four hearts reacting in this manner there was, during standstill, a slight but consistent rise in the threshold (from 0.99 to 1.06 volts) and a marked lengthening of chronaxie (from 4 to well over 6 sigmata). In this same ventricle, as in other cases, when the rate was kept constant, neither threshold nor chronaxie were at all affected by strong vagus stimulation. Figure 4 illustrates this type of effect.

A further report and analysis of the effects of change in ventricular rate upon rheobase and chronaxie is in progress and will appear later.

Comment. We had entertained some expectation that bathmotropic effects, not of vagus, but of sympathetic, origin might be encountered in these experiments, since it has been shown that sympathetic stimulation may relieve intraventricular block, and unpublished observations also pointed to the possibility of a bathmotropic influence. From our present observations, however, we are forced to conclude that if there are any effects of sympathetic fibers on the ventricle they are so slight as to escape detection by the method employed.

On the other hand, in view of the complete absence of vagus weakening of the ventricular contractions in the turtle, it is not surprising that we have failed to detect bathmotropic changes. In the auricles we have found that these changes are not apparent until there is a marked diminution in the height of contraction. Since no such change in contraction takes place in the ventricle, there is an *a priori* reason for doubting that changes in chronaxie occur except as an indirect result of vagus action.

SUMMARY

Stimulation of the neck vagus of the turtle produces no detectable change either in the rheobase or in the chronaxie of the ventricle if its rate is controlled. When changes do occur they are secondary to alterations in rate and are not due to intrinsic nervous influence. The data substantiate the conclusion that the ventricle is not innervated by the vagus.

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EXCITABILITY OF THE TURTLE AURICLE DURING VAGUS STIMULATION

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There is general agreement among investigators that vagus stimulation shortens the chronaxie of inhibited cardiac muscle (1), (2), (3), (4). Familiarity with experimentation is this field emphasizes the fact that deviations from this type result are met with, and the published reports reveal examples of variations which suggest that factors other than simple vagus inhibition have been contributory to the results obtained. In a previous communication dealing with the chronaxie of the turtle's ventricle we have reported the absence of vago-inhibitory influence on that structure, but showed that changes in cardiac rate induced by vagus stimulation or by other means often had a profound influence on the chronaxie (5).

Owing to the regional distributon of the vagi in the sinus and auricles, especially of the turtle heart, rate changes may be obtained which are no criterion of the inhibition elsewhere than in the sinus venosus. Our experiments have indicated that changes in the chronaxie due to vagus stimulation are not detectable in cardiac auricular muscle until there has been a very marked reduction in the force of contraction and it is imperative that this depression shall actually involve the tissue under the stimulating electrode. In view of these and other disturbing factors it is desirable that chronaxie measurements which are made with a view of determining the rôle played by the vagus shall be made under conditions which rigidly exclude fluctuations in physiological conditions other than those due to the inhibition and that precautions shall be taken to give assurance that the stimulated region actually will be depressed by the inhibitory action of the vagus. With this end in view it was decided to make the determinations of threshold and excitation time upon the quiescent auricle for comparison with those made during rhythmic contraction.

METHOD. The turtles were prepared as described in our previous communication, excepting that the auricular contractions were graphically recorded. In 9 of 24 experiments (each experiment including a large number of tests of excitability extending over a period of from 3 to 7 hours) on 13 turtles, a procedure employed by one of us was adopted to

render the auricle quiescent. A special heart-clamp, its jaws protected by gum tubing, was placed at the base of an auricle and gradually tightened until all rhythmic impulses were blocked, the appendage remaining quiescent. Under these conditions, if one has been careful not to over-compress the tissue, the vago-inhibitory fibers to the appendage will still conduct impulses and produce their typical inhibitory effects.

The condition of quiescence induced by clamping eliminates the fluctuating and disturbing effects of the rhythmic contractions and reduces the auricular appendix to a basic state of inactivity. This condition of arrest must not be confused with inhibition, for the arrested tissue responds vigorously to all excitatory agencies. Vagus stimulation produces inhibitory depression far below this resting level and the inhibitory effects can be determined with certainty and accuracy.

In 5 other experiments, each one as usual including a large series of tests, an approach to the condition of arrest was obtained, a slow rhythm being induced by cooling the sinus venosus. In these hearts the interval between beats was sufficient to permit complete diastolic rest which at least approximates the basal state of an auricle arrested by clamping. The rates of these hearts usually were still further slowed by the right vagus, but with left vagus stimulation they frequently remained unaltered (Fredericq and Garrey, 4).

Finally, all results were compared with 10 series of determinations made on auricles beating at a normal rhythm. In these cases care was taken to ensure application of each testing stimulus at the same phase of relaxation in the hope that the degree of recovery from activity in successive tests would always be the same, although it is doubtful whether this was usually true. In other respects the same precautions were observed as in the previously reported experiments on the ventricle.

One objection may be brought against the procedure of producing block between an auricular appendage and the rest of the heart. No matter how carefully the block is instituted, some vagus fibers will probably be blocked along with the muscle fibers. It may thus happen that the auricular tissue immediately beneath the stimulating electrode is not inhibited and will undergo no change in excitability. Some of our negative results may very well be a consequence of this condition. Even without clamping, however, the same factor may be present, for if vagus stimulation should be sufficiently strong to ensure direct inhibition of every auricular fiber, the amplitude of contraction would be so reduced as to make it impossible to detect or record the responses, if any should occur. Conversely, the region inhibited might be more profoundly inhibited than the remainder of the appendage. There is, however, no clear indication in any of our experiments that this ever happened.

RESULTS. Although, for the sake of emphasis, we have separated the

experiments into three groups on the basis of activity, the results, qualitatively, have been the same for each group. We shall, therefore, first give a general description of the vagus effects, leaving our presentation of these quantitative differences for later treatment.

Changes in threshold. In every case in which we report a change in threshold it must be understood that our interpretation is based upon observation of the consistent effect of vagus stimulation, never upon fluctuations, possibly due to uncontrollable influences, seen during only two or three periods of vagus stimulation. We have omitted one auricle

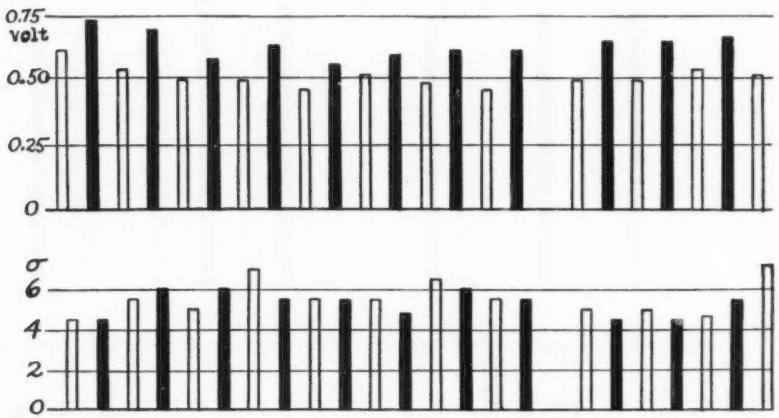

Fig. 1. Quiescent auricle, clamped at base. *Upper line*, rheobases. The height of each white column shows the rheobase, in volts, during a period when there was no inhibition; the height of each black column, the rheobase during a period of vagus stimulation. *Lower line*, chronaxies in sigmata. Again white and black indicate, respectively, absence or presence of inhibition. In each test the intensity used was double that of the previous rheobase. The tests both of rheobase and of chronaxie are given in chronological sequence, the longer gap toward the end indicating a long rest.

from consideration, although the vagus produced its typical effects, because vagus inhibition failed before an adequate series of tests could be completed. In every series of tests, both written and graphic records were kept, usually of every stimulus applied.

In 18 experiments out of a total of 24 on 13 hearts, the rheobase was raised by vagus stimulation, the increase ranging from 2 to 40 per cent. In some experiments the rheobase of the non-inhibited heart remained nearly constant over a long period of time (2 or 3 hours), or drifted slowly and progressively to a higher or lower level. Throughout this period vagus stimulation consistently raised that level, the degree of change being roughly proportional to the depth of inhibition (figs. 1 and 2).

With the possible exceptions noted below, vagus stimulation never produced a decrease in the rheobase, i.e., never increased the excitability. In one or two hearts there was some evidence, not very consistent, of a 2 to 4 per cent fall in rheobase during the periods of vagus stimulation before the amplitude of contraction had fallen to a constant level. In one of these hearts during the first two periods of vagus stimulation the rheobase

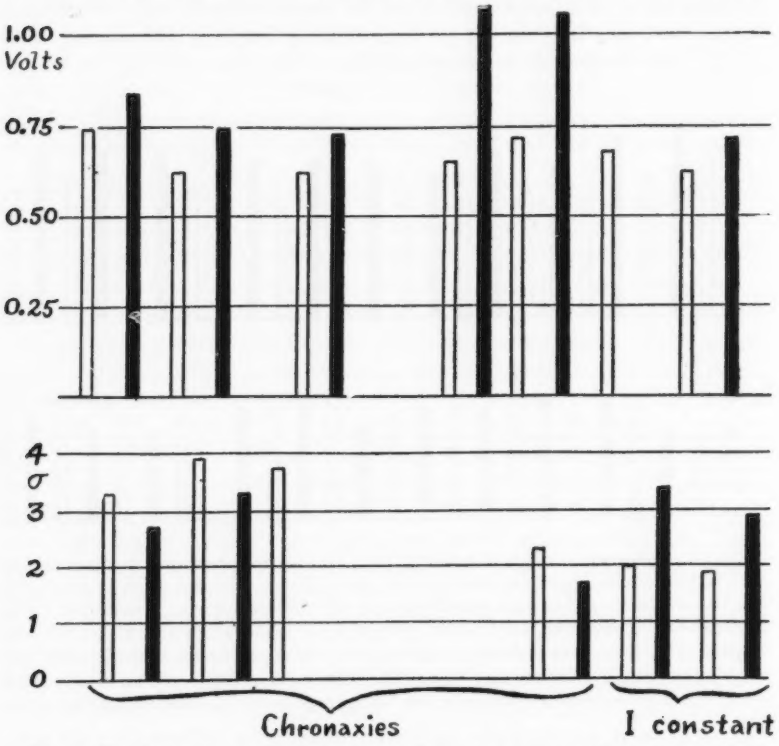

Fig. 2. Slowly beating auricle. Same as figure 1, excepting that at end of lower line the intensities used to determine least time were the same both with and without inhibition. These last four columns, therefore, are not chronaxies.

appeared to fall by about 22 per cent, but since this was at once followed by a violent apparent rise of 300 per cent after which other slighter irregularities appeared, we believe this exception may be disregarded.

Unless the inotropic influence of the vagus upon the auricle is considerable, the effect upon the rheobase may escape detection. A 50 per cent reduction in amplitude of contraction may produce a scarcely detectable change in excitability. In figure 3 we show the relation between the ino-

tropic and bathmotropic vagus effects for our 24 experiments. In the upper part of the figure the height of each column indicates the approximate average height of contraction during inhibition in terms of percentage of the height without inhibition. The experiments are arranged, not in chronological sequence, but in the order of increasing inotropic vagus effect. Immediately below each of these columns, in experiments where the vagus produced a change in rheobase, the extent of that change, expressed as percentage increase, is shown by another column. It is at once evident from the figure that only when the weakening of contraction becomes pronounced does the rheobase rise. In general, also, it is clear that the greater the fall is amplitude, the greater the rise in rheobase,

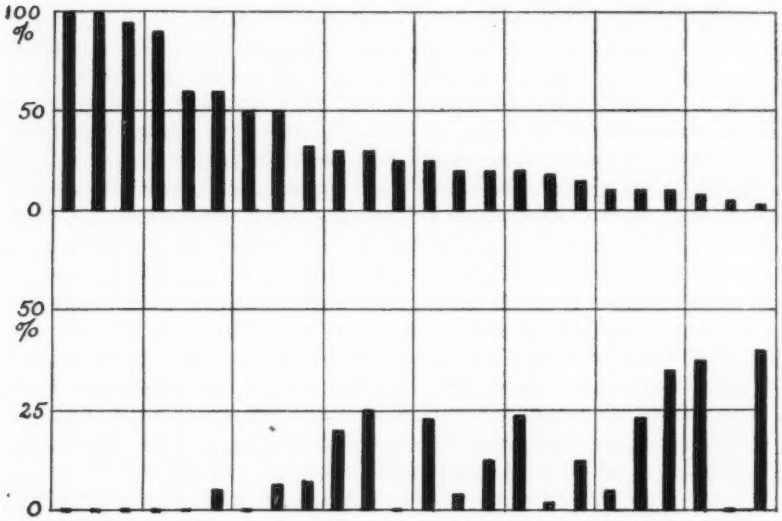

Fig. 3. The relation of rheobase to inotropic vagus effects. Described in text.

although considerable irregularities occur. For example, on one heart the contraction decreased 95 per cent, yet the rheobase rose not at all.

As numerous observations demonstrate, this relation between inotropic and bathmotropic effects is shown much more clearly by the individual auricle; yet even here the correlation between strength of contraction and excitability is not absolute. It is just such factors as variation in vagus effectiveness during long periods of time, and perhaps the deleterious effect of repeated testing stimuli, that prevent figure 3 from being quantitatively accurate. Frequently neither rheobase nor amplitude remained sufficiently constant over a long enough period to enable one to arrive at much more than a rough average of the general effect.

The fact that a 50 per cent reduction in amplitude is associated with little or no change in rheobase may well mean that the inotropic change is due to the greater inhibition of some muscular fibers than of others, the relatively uninhibited fibers remaining normally excitable. When, however, the inotropic influence becomes greater, the effect of inhibition upon the rheobase becomes greater. We have no doubt that, in some of the hearts tested, if the rheobasic level could have been measured after the disappearance of recordable contractions, the level would have been raised by more than 100 per cent. In a few tests, in fact, changes of approximately this magnitude were observed. Why rheobasic changes are sometimes absent has already been discussed in the final paragraph under method, and will be referred to again.

Interpretation of the results is further complicated by the fact that vagus stimulation can produce intra-auricular block in the turtle, and for this reason one might argue that the vagal effects we observed were, in reality, not due to decreased excitability, but that the impulses set up in the immediate vicinity of the stimulating electrode by weaker stimuli did not reach the surrounding muscle because of block. Stronger stimuli, affecting a larger area, may have elicited impulses which were not blocked. Although it is impossible to prove that this is not the explanation, it appears unlikely when it is considered that the vagus not only raises the threshold but also increases the required time for stimuli above threshold strength. In any event, it is difficult to believe that block will appear in the absence of a rise in threshold.

Changes in chronaxie. Our data upon chronaxie are not so complete as upon changes in rheobase, partly because reliable chronaxies are much more difficult to obtain. The rheobase, although raised, will only very rarely remain constant for as long as a minute during vagus stimulation, and that is too short a time properly to determine a rheobase, a chronaxie, and another control rheobase which must agree with the first if the chronaxie determination is to be accepted. If one attempts to utilize three successive periods of vagus stimulation, it is not often that the two rheobases will agree, and even when they do agree the total time consumed is so great as to throw doubt upon the result. The other reason for paucity of chronaxie measurements is that we laid much more emphasis upon intensity-time determinations with fixed intensities to be described below, since we felt they were far more significant as an index of excitability.

In spite of the difficulties, we have a number of determinations of chronaxie which we regard as reliable, and in several hearts the number of measurements is so large that, in spite of fluctuations, there can be no doubt of the result. In one heart, for example, vagus stimulation, although raising the threshold from 0.49 to 0.59 volt, shortened the chronaxie from 5.6 to 5.3 sigmata; in another heart the rheobase rose from 0.52 to

0.69 volt and the chronaxie decreased from 7.5 to 6.1 sigmata. In two other hearts the chronaxie was definitely shortened and in three others the evidence for shortening was good. It must be stressed that only in those hearts which show a considerable increase in rheobase is the change in chronaxie sufficient to be detectable. Where there is no change in rheobase there is apparently never a change in chronaxie. In other words, in our experience, the vagus decrease in chronaxie is always associated with an increase in the threshold (figs. 1 and 2).

Intensity-time relations other than chronaxie. We have an abundance of data regarding the effect of vagus stimulation upon the least time required for excitation by a current of constant strength. Generally the strength selected was double the rheobase of the noninhibited auricle. With two or three very doubtful exceptions, vagus stimulation always increased the required time, provided the rheobase is also raised. If the rheobase is not influenced by inhibition, the least time is likewise unaffected. Unlike determinations of chronaxie, it is very easy to choose an intensity, more or less arbitrarily, and make a number of alternate determinations of least time during vagus inhibition or without inhibition. Detection of the time difference is easiest when the strength chosen is not greatly in excess of the rheobase, for, with increasing intensities, not only the absolute, but also the relative increase in least time becomes smaller and, therefore, harder to measure. Obviously the repeated measurements serve as controls of each other, and changes in excitability are as easily, and even more satisfactorily, detected by this means as by measurement of the rheobase. Most important is the fact that they very strongly support the evidence for vagal depression of excitability derived from rheobasic determinations. In figure 2 a few least time measurements are given. As in figure 1, the black columns mean vagus stimulation; the white columns signify absence of such stimulation. The least time in each trial is indicated by the height of the column in the lower series.

In a number of auricles we also determined the relation of intensity to least time over a considerable range of intensities, both for the inhibited and for the uninhibited muscle. An example is given in figure 4. It will be noted that the curves obtained during vagus stimulation are not quite of the same form as those obtained without such stimulation. The curves tend to converge at their left extremities. The consistent shortening of chronaxie produced by vagus stimulation is a reflection of this convergence.

Comparison of results on the quiescent, slow, and rapidly beating auricle. The only observed difference under these three conditions was one of frequency of appearance of demonstrable changes in excitability resulting from vagus stimulation.

In all, 9 auricles were clamped so that the appendage was either quiescent or beating very slowly. In 2 of these there was no inotropic effect of

the vagus, both muscle and nerve fibers having been blocked, and, consequently, there were no vagal effects upon excitability. In 2 other appendages, vagus stimulation produced no change in threshold in spite of a considerable inotropic effect. We have already suggested the probable reason for this finding, namely, that some fibers in the immediate neighborhood of the stimulating were uninhibited. In the remaining 5 auricles of this group, the increases in rheobase were respectively 4, 5, 6, 23 and 35 per cent. The characteristic changes in chronaxie and in least time were seen in this group.

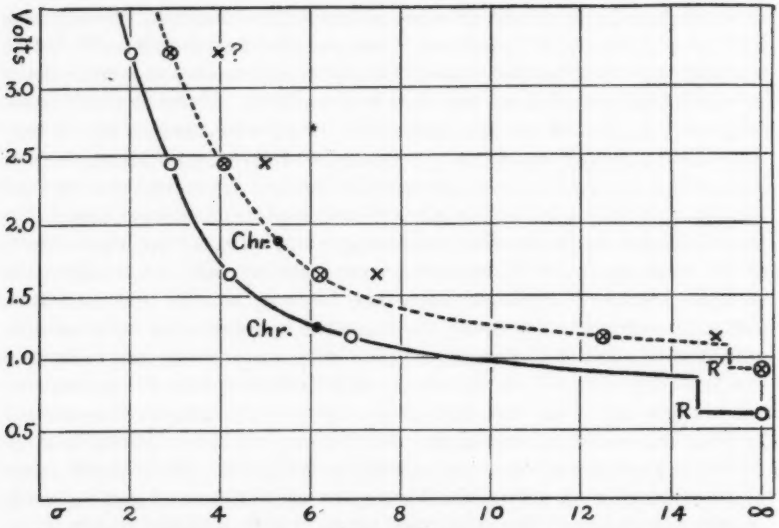

Fig. 4. Quiescent auricle. Curves relating intensity and time; ○, auricle uninhibited; ⊗, average curve during moderate inhibition; ×, during stronger inhibition which all but abolished contraction. *R* and *R'* indicate rheobasic intensities. No rheobase was determined during the strongest inhibition. *Chr.* indicates position of chronaxie, not actually determined.

Only five auricles were caused to beat slowly by cooling the sinus, but in every one of these auricles a marked vagus effect was demonstrable, either together with or without further slowing from vagus stimulation. The increases in rheobase in the several auricles were 12.5, 23, 24, 25, and 40 per cent, and again there appeared the characteristic changes in chronaxie and least time. The quantitative difference between this group and the former one, if not due to chance, is attributable to the clamping in the first group which must have blocked many vagus fibers in most of the experiments. The difference was not due to the age of the preparation.

Finally, 10 experiments were carried out on hearts beating at a moderate or rapid rhythm. The left vagus was usually stimulated and produced definite slowing in only 4 or 5 experiments. Usually, too, the left auricle was tested. In 3 of these auricles, with 40, 50 and 75 per cent reductions in amplitude, no change in excitability was observed during vagus stimulation. In the 7 remaining experiments the rheobasic changes were 2, 5, 6, 6, 18, 20 and 20 per cent respectively; and again the typical influence upon least time and chronaxie was observed. In contrast with the second group, the presence of a rapid rhythm appears to diminish the vagus effect.

The effect of acetyl choline. The influence of this drug was tested on three hearts only, but was in all respects similar to that of strong vagus stimulation. In all three experiments the rheobase was greatly increased. The chronaxie, untested in one experiment, was shortened in the other two. Least time was not determined, but there can be no doubt that it would have been increased. In one experiment, when the application of the choline solution was preceded by Ringer solution of approximately the same pH to obviate the possible effects of moistening the stimulating electrode and of the hydrogen ion, the rheobase was increased from 0.75 to 1.40 volt and the chronaxie was reduced from 2.75 to 1.5 sigmata. That the chronaxie measurements, repeated at intervals during recovery, are relatively reliable in this experiment is shown by the fact that the value had again risen to 2.25 sigmata when the rheobase had later fallen to 0.85 volt. Following the application of acetyl choline the excitability gradually returned to nearly the original value and, as already said, the chronaxie again increased.

The effect of atropine. This was tried only once on a slowly beating auricle, but the effect was most striking. Shortly before the application the auricle was washed with Ringer solution, and following, this stimulation of the vagus, as previously in the same heart, raised the rheobase, now more than 100 per cent. The actual sequence of rheobasic changes after the addition of the Ringer solution was as follows. No vagus stimulation, rheobase 1.12 volts; vagus stimulated, rheobase about 2.50 volts; vagus at rest, 0.99 volt. Atropine sulphate solution was now applied and the vagus stimulated at suitable intervals to demonstrate the progressive paralysis of the vagus terminations with the corresponding elimination of the effects of the vagus upon the rheobase, as follows. Vagus stimulation, rheobase 1.44 volt; no stimulation, 0.70 volt; stimulation, 0.80; no stimulation, 0.70; stimulation, 0.70; no stimulation, 0.70; stimulation, 0.70. The decrease in the inotropic action of the vagus, at first causing at least a 95 per cent reduction in amplitude, ran parallel with its decreasing effect upon the rheobase. During the last two periods of stimulation the reduction in amplitude was barely perceptible and was no doubt due to the weakening of some basal region of the auricle not yet reached by the atropine solution. The chronaxie was not tested in this case.

DISCUSSION AND CONCLUSIONS. All doubt that the changes in excitability during vagus stimulation are really associated with inhibition in the region of the stimulating electrode, and are not due to any other factor, such as sympathetic nerve effects, should be dispelled by the following recapitulation of the evidence.

1. The changes in excitability, particularly in the individual heart, are roughly proportional to the inotropic effect of vagus stimulation.

2. They are obtainable in the quiescent auricle when such factors as change in rate and associated changes in metabolic activity and refractory period are rigidly excluded.

3. The same changes in excitability are produced by acetyl choline.

4. They are abolished by atropine.

5. They are not observed in the ventricle, which, almost certainly, is not directly inhibited, but merely arrested, by vagus stimulation.

It will have been observed that our findings are in agreement with those reported by others in that vagus inhibition shortens the chronaxie of the auricle. But, unlike the reported measurements of other investigators, we find that the shortening is always associated with a raised threshold, and we have not observed it along with an unchanged or even lowered rheobase. For this disagreement we have no fully satisfactory explanation, although several possibilities suggest themselves. First, there is vagus slowing, present in some previous investigations, controlled in ours. In view of the reported finding that the vagus diminishes lactic acid production in the inhibited heart while leaving the rate of its removal unaffected, one effect of inhibition of the beating auricle, even in the absence of chronotropic changes, will be the equivalent of rest. Under certain conditions this might account for diminished chronaxie and lower rheobase as reported for the inhibited frog ventricle by Field and Brücke (3). Another factor to be considered is vagus shortening of the absolutely refractory period. Thus, even if the interval between the beginning of auricular systole and application of the testing stimulus be kept constant, the degree of recovery from refractoriness cannot be assumed to be the same. However, even when full allowance is made for these factors, factors which could have played no part in our experiments with the quiescent auricle, the discrepancies between our results and those of others are not satisfactorily accounted for. A third factor may very possibly be the degree of inhibition at the site of stimulation, as we have already emphasized.

In conclusion the general question must be asked: What is the effect of vagus stimulation upon the excitability of the auricular muscle of the turtle heart? The answer given by some students states that the excitability is increased because the chronaxie is shortened. Changes in rheobase are wholly disregarded because consistent changes were not found, and the

chance fluctuations were conceived to be of such magnitude as to render the measurements valueless.

On the basis of our results, however, we believe the proposed question can be answered unequivocally. If we define excitability in terms of the energy required to elicit the response, then vagus stimulation *decreases* the excitability; for inhibition not only increases the current intensity required to excite, but it also increases the time required for excitation by a stronger current of fixed strength. At every intensity used, inhibition increases the quantity of electrical energy required for excitation. Thus our conclusion regarding the effect of vagus stimulation upon the excitability of the inhibited heart muscle is the same as that given by Schiff in 1850, and confirmed by a long line of later observers.

A shortened chronaxie alone is thus no criterion of increased excitability. Its occurrence in these experiments simply means that the curves relating intensity to least time are not of the same form in the inhibited and in the uninhibited auricle.

SUMMARY

1. Measurements of changes in rheobase, in chronaxie and in "least time" produced by vagus stimulation were made on the turtle auricle which was either rendered quiescent, slowed, or allowed to beat at a normal rhythm.

2. Providing the region in contact with the stimulating electrode is directly inhibited and not merely arrested as a result of the cessation of activity elsewhere, vagus stimulation produces a definite rise in the threshold (rheobase) of excitation by the constant current; a slight or moderate shortening of the chronaxie, which is always associated with a rise in the rheobase; and a lengthening of the time required for excitation by a current of greater than rheobasic strength.

3. The effects of acetyl choline are the same as those of vagus stimulation.

4. Atropine eliminates the vagus effects on excitability.

5. In one and the same heart the magnitude of the observed changes in excitability is roughly proportional to the degree of inhibitory weakening of the systoles.

6. When different hearts are compared, the same fact holds, with some exceptions.

7. The changes in excitability were never observed in the absence of conspicuous inotropic action of the vagus on the auricle tested.

8. If excitability be defined in terms of the quantity of energy required for excitation, then vagus stimulation decreases excitability.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

XXXI. Effects of Anterior Pituitary Hormones on Gonads and Other Organ Weights in the Pigeon

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In an earlier study Riddle and Flemion (1928) found that the intraperitoneal injection of a particular anterior pituitary extract into immature ring doves caused a very marked growth in the testes and much less growth in the ovaries; this is the reverse of the relative response of testis and ovary in mammals. The transplantation of anterior lobe substance of mature doves into immature ones gave similar though much less pronounced results on gonad-stimulation. It was also found that birds receiving the effective gonad-stimulating extract usually had enlarged thyroids, livers and spleens, though the other treatments (transplants, etc.) employed were probably without effect on the weights of these organs. During the past decade the relation of the anterior pituitary to the development, compensatory hypertrophy and enlargement of the thyroid has received the attention of several investigators (Loeb, Aron, Gray), and the further study of organ weights now reported have a bearing on this pituitary-thyroid relationship. The present data relate to weight changes of various organs of immature birds treated with a variety of pituitary extracts.

It can now be shown that the immature bird testis is extraordinarily responsive to the maturity hormone; that the growth response of the immature dove and pigeon ovary to the sex maturity hormone is not more than one-third or one-fourth of that of the testis; that alkaline anterior lobe extracts containing both growth and maturity principles produce a prompt and marked enlargement of the thyroid and liver, though most other organs undergo little or no change in size; that the luteinizing hormone from pregnant urine does not increase thyroid size, does diminish the oviduct, and probably stops or retards growth in the ovary; and that posterior lobe extracts administered over similar short periods probably do not affect the size of any of the organs studied.

BIRDS AND PREPARATIONS USED. Only quite immature birds, though of nearly full or final body weight, were used in this study; it could thus be known that at the beginning of dosage the gonads were of very small and essentially predictable size, and that they were still undergoing a very slow rate of growth (Riddle, 1928). Ten somewhat older doves (aged 3.4 to 3.9 months) have been properly included in the data of table 3, but excluded from the data concerning gonad-stimulation recorded in tables 1 and 2. Some common pigeons of 3.2 and 3.3 months are included in table 2, but it should be noted that the testes of some birds of this age may have begun their period of rapid growth independently of the treatment. Most birds were killed for examination at about 75 days after hatching; since age in these animals is more properly reckoned from the beginning of embryonic development our tables record these ages as at or near 3.0 months. The most favorable age for testing the maturity hormone lies between 1.5 and 2.2 months after hatching. Various races of ring doves and of pigeons were used, and these races—particularly among the pigeons—are of unequal body size and show differences in average age of attaining sexual maturity. It therefore follows that the "control" birds of one group are not necessarily the equivalent of other controls. In general the control for each test animal was a brother or sister, and of the same age, as the test animal. In a few cases the control is not of the same fraternity, but of the same inbred strain. Also, the same control bird sometimes serves as control at more than one point in the tabulation. The controls were injected daily with NaCl solution (or glycerin).

The commercial pituitary extract of Lang is dissolved in glycerin, and the control birds for this particular series of tests were injected with glycerin only. It is known that this extract is prepared from fresh bovine anterior pituitaries, and that it contains the gonad-stimulating hormone in very large amounts; it was used here intraperitoneally only. The "growth" hormone (from anterior lobe of cattle), "sex maturity" (from whole pituitary of sheep) and "luteinizing" (from pregnant urine) hormones we obtained through the courtesy of the Research Laboratories of Parke, Davis and Company. The method used in preparing the growth principle is essentially that of Putnam, Teel and Benedict (1928, 1929), slightly modified by Bugbee, Simond and Grimes (1931). The sex principle was also an alkaline extract obtained by methods described by Bugbee, Simond and Grimes, and rather similar to those employed by Evans and Simpson (1928) and others for the extraction of the growth hormone. The data of tables 1 and 2 show that the growth hormone extracts used by us contained also the maturity principle. It is probable that the sex maturity extracts likewise contained more or less of the growth hormone. The luteinizing hormone, obtained from pregnant urine, contained 50 R. U. per cubic centimeter. All the above-mentioned extracts were fresh preparations kept throughout at low temperatures. The antuitrin, pitocin (alpha-hypophamine) and pitressin (beta-hypophamine) used were the commercial (fresh) preparations. All extracts (except that of Lang) were injected into the pectoral muscles.

The same pituitary preparations, and practically the same group of birds used in the present study, were also utilized by Riddle and Braucher (1931) in testing the source of the pituitary hormone found by them to regulate the production of "crop-milk" in the pigeon. The data fully tabulated here incidentally provide the evidence that the crop-gland response was there obtained from only those pituitary preparations which also produced marked growth in gonads and enlargement of thyroid and liver.

TABLE 1

The gonad-stimulating effect of various pituitary extracts in ring doves

ANTERIOR PITUITARY EXTRACT	SEX	DURATION OF TREATMENT	TREATED OR CONTROL (T OR C)	AGE OF BIRD	WEIGHT		PER CENT GONAD INCREASE PER DAY TREATED
					Gonad	Oviduct	
		days		*months*	*mgm.*	*mgm.*	
Commercial (Lang) (intraperitoneal)	Males	9	T	3.1	132.6		144.0
			C	3.1	9.5		
		3	T	3.1	24.4		61.2
			C	3.1	8.6		
		10	T	3.0	190.2		227.7
			C	3.0	8.0		
		11	T	3.0	73.1		52.4
			C	3.1	10.8		
		3	T	3.0	20.7		47.8
			C	3.0	8.5		
		10	T	2.7	140.0		235.6
			C	2.7	5.7		
	Females	11	T	3.2	42.5	79.6	6.4
			C	3.2	24.9	31.5	
		3	T	3.1	30.3	34.3	14.3
			C	3.1	21.2	23.2	
		3	T	2.9	14.2	14.5	−3.6
			C	2.9	15.9	16.7	
Maturity hormone	Males	4	T	3.1	36.4		23.1
			C	3.2	18.9		
	Females	5	T	2.8	36.8	(27.7)	24.6
			C	3.0	16.5	23.1	
Growth hormone	Females	5	T	2.9	31.5	13.4	31.2
			C	3.0	12.3	13.6	
		4	T	2.9	15.0	23.3	3.0
			C	3.0	13.4	14.1	
Antuitrin	Males	3	T	3.0	39.0		103.5
			C	3.0	9.5		
		3	T	2.9	16.3		30.6
			C	3.1	8.5		
		5	T	2.7	16.1		25.4
			C	2.6	7.1		

TABLE 1—*Concluded*

ANTERIOR PITUITARY EXTRACT	SEX	DURATION OF TREATMENT	TREATED OR CONTROL (T OR C)	AGE OF BIRD	WEIGHT		PER CENT GONAD INCREASE PER DAY TREATED
					Gonad	Oviduct	
		days		*months*	*mgm.*	*mgm.*	
Antuitrin—*Concluded*	Females	4	T	3.2	20.8	14.8	−6.6
			C	3.1	28.3	31.3	
		5	T	2.6	15.4	13.4	4.2
			C	2.6	12.7	12.2	
	Males	6	T	3.0	7.9		−4.4
			C	3.0	10.7		
		5	T	2.9	9.2		?0.0
			T	2.9	8.2		?0.0
Luteinizing hormone (from urine)	Females	7	T	3.0	30.0	18.2	1.1
			C	3.0	27.9	35.2	
		5	T	3.0	21.9	11.0	−4.5
			C	3.1	28.3	31.3	
		5	T	2.9	9.3	11.2	−4.9
			C	3.0	12.3	13.6	

RESULTS. *The relative effect of the maturity hormone on testis and ovary.* The data on this point are given in tables 1 and 2. It is clear that the three different anterior lobe extracts used—that of Lang, the growth, and the maturity—all contained the gonad-stimulating principle. All of the 13 tests made on males with these extracts showed a stimulation of testis growth of from 23 to 235 per cent per day of treatment; 11 of 12 treated ovaries showed an increase of from 3 to 31 per cent—1 giving an indicated decrease (or failure to grow during treatment) of 3.6 per cent. The duration of treatment was quite unequal among the different groups of birds, and it is evident from the tables that the longer periods of dosage (9–11 days) show much higher percentage increases per day of treatment. It is therefore incorrect to compare the percentage increase in long-term treatment with the percentage increase under short-term treatment. The testes of 4 males treated 9–11 days showed the phenomenal average increase of 165 per cent per day; the ovary of 1 female, an increase of only one twenty-fifth this amount (6.4 per cent). The testes of 9 males treated 2–6 days showed an increase of 46 per cent per day while the ovaries of 9 such females increased at a rate less than one-third as great (13.9 per cent). We believe these data largely justify the conclusion that in doves and pigeons a definite large quantity of the gonad-stimulating

TABLE 2

The gonad-stimulating effect of various pituitary extracts in common pigeons

ANTERIOR PITUITARY EXTRACT	SEX	DURATION OF TREATMENT	TREATED OR CONTROL (T OR C)	AGE OF BIRD	WEIGHT		PER CENT GONAD INCREASE PER DAY TREATED
					Gonad	Oviduct	
		days		*months*	*mgm.*	*mgm.*	
Commercial (Lang)	Females	3	T	3.0	51.5	43.0	9.3
		2	T	3.0	57.0	52.3	20.7
			C	3.1	40.3	35.3	
Maturity hormone	Males	5	T	2.9	156.9		59.3
		6	T	2.8	177.9		58.2
		4	C	3.3	40.9		
		7	C	3.1	38.2		
	Females	4	T	3.3	70.0	40.4	19.8
		7	C	2.8	39.1	36.6	
		6	T	2.9	33.0	22.9	5.7
		3	C	2.8	24.6	24.3	
Growth hormone	Males	4	T	3.3	208.3		64.8
			C	3.2	58.0		
		5	T	2.9	81.4		22.6
			C	3.1	38.2		
		6	T	2.8	173.1		57.5
		6	T	2.8	92.3		22.9
		5	C	2.9	38.7		
			C	2.8	39.1		
Antuitrin	Males	4	T	2.3	37.4		15.8
			C	2.3	22.9		
	Females	3	T	2.4	22.4	24.2	?0.0
		3	T	2.3	22.3	19.8	?0.0
Luteinizing hormone (from urine)	Males	5	T	3.2	182.5		67.1
			C	3.1	41.9		
		4	T	3.1	32.1		−4.0
		3	C	3.1	38.2		
		7	T	3.0	51.7		3.3
		3	C	3.1	41.9		
	Females	4	T	3.2	47.4	41.9	−2.3
		4	T	3.0	38.0	28.0	−6.8
		3	C	3.0	52.3	49.4	
		5	T	3.2	39.8	29.9	0.4
			C	2.8	39.1	36.6	

TABLE 2—*Concluded*

PITUITARY EXTRACT	SEX	DURATION OF TREATMENT	TREATED OR CONTROL (T OR C)	AGE OF BIRD	WEIGHT		PER CENT GONAD INCREASE PER DAY TREATED
					Gonad	Oviduct	
		days		*months*	*mgm.*	*mgm.*	
Pitocin	Males	3	T	3.2	40.3		?0.0
	Females	3	T	3.1	49.4	18.6	?0.0
Pitressin	Males	3	T	3.3	62.5		10.0
		4	T	3.3	40.3		−4.1
		3	C	3.2	58.0		
		3	C	3.1	38.2		
		4	T	3.1	41.4		?0.0
Pitocin—Ring dove	Males	2	T	3.0	22.2		8.7
			C	3.2	18.9		
	Females	3	T	3.0	15.2	33.4	−1.5
			C	2.9	15.9		
Pitressin—Ring dove	Males	3	T	2.9	5.5		−14.0
			C	3.0	9.5		
	Females	4	T	3.1	16.6	18.5	6.0
			C	3.1	13.4	14.1	

hormone produces at least three or four times more gonad growth in the male than in the female. It is interesting to note that this is also approximately the ratio existing between the ultimate or adult weights of the two kinds of gonads—the testes being 3–5 times heavier than the (resting) ovary in these animals.

The oviducts of treated females reflect this gonad-stimulating effect of these extracts, though less definitely; 9 of the 12 treated oviducts are larger, and three are very slightly smaller than their respective controls.

The presence in antuitrin of the sex maturity principle, in amounts detectable by a test on the immature bird testis, is of interest. Four such tests all show an increase in testis size—varying between 16 and 103 per cent per day. The concentration of this principle seems, however, too weak to yield a definite result in females (4 tests). The posterior lobe principles—pitocin and pitressin—give no evidence whatsoever (table 2) of gonad-stimulating power.

Adverse effects of luteinizing hormone. The luteinizing hormone, as obtained from pregnant urine, is shown in 12 tests to produce no gonad growth; on the other hand, when age differences of treated and control

are here taken into account (the large testis of the pigeon aged 3.2 months may have begun rapid growth before treatment), the data clearly suggest that gonad development was *retarded* by this hormone. This antagonism to growth in the sexual apparatus is particularly marked in the case of the oviduct, which was smaller in all of the 6 tests than in the control. The extract used produced an evident luteinization in all of the 6 tests. The wholly different nature of the action of luteinizing and sex maturity hormones is clearly shown by these tests.

Effects of various pituitary hormones on the size of various organs. The data are given in table 3. Prolonged heavy dosage with the commercial product of Lang was plainly accompanied by a marked increase (nearly double) in the size of the suprarenals; this effect is not evident in short-term dosage. Our results make it entirely probable that the prolonged intra-abdominal administration of glycerin only (to the control) caused some suprarenal enlargement. None of the other hormones used produced notable change in suprarenal size during the relatively short periods tested.

The weight of the thyroid and liver was markedly increased, in both doves and pigeons, by the administration of either of the three preparations (Lang, growth, and maturity) which were proved to have a strong gonad-stimulating effect (probably all contained the growth principle also). In the case of all these extracts it is of course possible that impurities not of hormonal nature may have been responsible for this result. Schockaert (1930) noted a doubling of thyroid size in ducks injected with lactalbumen, turpentine, etc. But some aspects of responsiveness of the thyroid to anterior pituitary stimulation are known, and it seems more probable that this weight increase is a response of the thyroid to a large excess of its pituitary stimulator. Our data indicate clearly enough that these effects on thyroid and liver are not due to such contaminating substances as are contained in antuitrin, pitocin, pitressin, and the luteinizing preparation used. These extracts increased the size of neither the thyroid nor the liver. Moreover, the glycerol-alkaline extract of Korenchewsky (1930), which lacked both growth and gonad-stimulating properties, is said to have caused a reduction of thyroid size in rats.

The body weight increased during treatment in all of the 6 birds given the growth hormone. A slight loss of weight characterized all other groups, including the (injected) control; this loss was least notable in the group receiving the maturity hormone which, as already noted, probably contained also some of the growth principle. Weights or measurements of spleen, heart and intestine fail to show any consistent effect of any of the hormones used. Thymus and bursa weights were diminished under probably all treatments excepting those with growth and maturity hormones; but such decreases are of no real significance here since they seem

TABLE 3

Average weights of adrenals, thyroids and livers in doves (above) and pigeons (below) treated with pituitary extracts; with control

PITUITARY HORMONE AND DOSAGE			BODY WEIGHT	ADRENAL	THYROID	LIVER	NUMBER OF BIRDS
Kind	Daily volume	Duration					
Doves							
	cc.	*days*	*grams*	*mgm.*	*mgm.*	*grams*	
Commercial (Lang)	0.3	9–11	150	19.3	50.7	8.96	8
Control	0.3 (glycerin)		145	12.7	32.8	3.84	8
Commercial (Lang)	0.4	2–3	135	10.9	35.9	4.97	4
Control	0.4 (glycerin)		137	10.8	21.6	3.07	4
Growth	0.3	2–9	142	10.2	42.4	4.49	2
Control			125	10.4	15.6	2.11	2
Maturity	0.4	4–5	141	11.5	22.7	3.59	2
Control			131	9.4	15.0	2.70	2
Antuitrin	0.1–0.5	3–5	133	10.2	29.6	2.56	5
Control			134	9.2	33.0	2.47	4
Pitocin-pitressin	0.1–0.3	3–4	134	11.5	20.6	2.55	4
Control			126	11.3	23.9	2.94	3
Luteinizing	0.2–0.4	4–7	138	9.6	25.1	3.08	8
Control			138	9.6	21.2	2.50	6
Pigeons							
Commercial (Lang)	0.4	2–3	330		87.9	10.14	2
Control	0.4 (glycerin)		312		72.3	6.45	3
Growth	0.8	4–6	318		65.8	12.69	4
Control			320		42.8	6.47	3
Maturity	0.4–0.8	4–6	316		63.7	8.60	4
Control			303		37.6	5.20	3
Antuitrin	0.7	3–4	333		64.9	6.33	3
Control			283		54.0	5.60	2
Pitocin-pitressin	0.1–0.15	3–5	322		72.9	5.94	5
Control			299		45.6	6.53	5
Luteinizing	0.2–0.8	4–7	310		54.6	6.74	6
Control			304		56.0	5.43	5

to accompany most conditions which adversely affect the body weight. Data concerning possible size changes in the pituitaries of the treated birds are reserved for later consideration.

In their early report on effects of intraperitoneal administration of fresh anterior lobe substance to rats Evans and Long (1922) noted no change in weight of the hypophysis, thyroids and thymus. After implantation of hypophyses in female guinea pigs Watrin and Florentin (1929) noted an increase of thyroid size which was attributed to an increase of folliculin following the hypophyseal stimulation of the ovary. Our tests make this explanation highly improbable since this response of the thyroid occurs equally in males and females.

SUMMARY

Tests were made of the effects of various pituitary hormones on weights of gonads and several other organs in 57 birds; 39 blank-injected birds were used as controls.

The immature testis of the dove and pigeon provides an extraordinarily sensitive test for the sex maturity hormone; 10 daily doses yield weight increases of from 500 to 2200 per cent; 3 daily doses give increases of more than 100 per cent.

The growth response of the ovary of the immature dove and pigeon is not more than one-third or one-fourth that observed in the testis.

Alkaline extracts of the anterior pituitary which were rich in the maturity principle, and probably contained the growth hormone, produced prompt and marked increase of weight in thyroid and liver; most other organs showed little or no change in size.

Antuitrin is capable of stimulating growth in the immature testes; but in similar dosage and short-term tests it gives no measurable response in the ovaries.

None of these results is ascribable to contamination of the extracts by posterior lobe principles; under the conditions observed in these tests pitocin and pitressin do not affect the size of gonads, thyroids or liver.

The luteinizing hormone from pregnant urine has no effect on size of liver or thyroid, but it decreases the size of the oviduct (uterus) and stops or retards growth in the ovary. Its action is distinct from, perhaps antagonistic to, that of the maturity principle.

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DOES HEMOPHILIC BLOOD CONTAIN AN EXCESS OF AN ANTICOAGULANT?

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The definition of hemophilia given by Bulloch and Fildes (1), namely, that it is an "inherited tendency in males to bleed" may be amplified to read: Hemophilia is a condition characterised by a delayed clotting time of the blood and a history of repeated bleedings, which is found only in males and is transmitted as a sex-linked character. The belief that the gene or genes conveying the defect occur in the sex chromosomes gives a theoretical explanation of the law of Nasse, that is to say, the fact that a hemophilic male transmits his defect, not to his sons, but to some of his grandsons, through his daughters who act as so-called conductors.

Experimental work upon hemophilia has not been concerned with the cause of the genetic defect, but with an explanation of the outstanding symptom, the delayed clotting time of the blood. All of the various factors of coagulation have been studied, and it may be said that there is pretty general agreement that the fibrinogen, the calcium, the prothrombin and the thromboplastic substance or cytozyme of the hemophilic do not vary from the normal. Weil (2) in 1905 first suggested that the delayed coagulation might be due to the presence of an anticoagulant. This view was tested by himself and other observers by various methods. While contradictory results have been reported the balance of evidence is clearly against this explanation. There are two known anticoagulants in the blood, namely, antithrombin and antiprothrombin (heparin). With regard to the former several observers, Nolf and Herry (3), Addis (4), Howell (5), Minot and Lee (6), who have made special examinations, report negative results. Howell (7) has made a negative report also in the case of antiprothrombin (heparin). He was unable to show any increase of this substance above normal in the blood of hemophilics. Quite recently, however, Fuchs and Falkenhausen (8) have revived this theory. They believe that in the hemophilic blood, as in the incoagulable blood of a peptonized dog, there is an excess of antiprothrombin which suffices to explain its prolonged clotting time. In the paper quoted they give no

direct evidence for this view, but support it rather by reference to certain similarities in the behavior of hemophilic blood and the blood of a peptonized dog.

Fuchs (9) has recently made use of a new method, devised by Barratt (10), for obtaining antiprothrombin (heparin) from small amounts (5 cc.) of blood plasma. The present authors have undertaken to use this method upon normal and hemophilic blood to ascertain whether or not the latter contains a larger amount of the antiprothrombin. The method as modified by Fuchs comprises the following steps (the slight changes from the original which were made are given in brackets).

1. Blood is caught from an artery in oxalate solution and centrifugalized. Five cubic centimeters of the clear plasma are pipetted off.

2. Have prepared 45 cc. of cold distilled water, and 450 cc. of boiling distilled water. Add the 5 cc. of clear plasma to the 45 cc. cold water, stir well and pour into the boiling water; remove at once from the heat and cool rapidly to 60°C.

3. Evaporate this mixture, under low pressure, to about 20 cc. The special apparatus used for this purpose is illustrated in the original article. During this process the temperature must not exceed 60°C. Practically this end is obtained by keeping the pressure below 148 mm. Hg.

4. The 20 cc. of material, together with the distilled water used for washing out the flask are dialyzed against running distilled water for six hours. (In this work it was dialyzed against running tap water all night, and against two changes of distilled water (500 cc.) for half an hour each.)

5. Evaporate the dialyzed liquid to dryness over a water-bath.

6. Grind the residue in a mortar and extract thoroughly with 20 cc. of distilled water, six times. The ground material is put in a strong flask with glass beads and 20 cc. distilled water, and shaken in a machine for half an hour. (In this work the residue was ground with clean sand, and the sand and powder put in a large test-tube and shaken with distilled water for half an hour. The material was sedimented in the centrifuge at the end of each shaking, and the clear extract poured off.) The six extracts are combined and evaporated to dryness on the water-bath.

7. The residue is extracted with 20 cc. distilled water, filtered, and the filtrate again evaporated to dryness on the water-bath. This process is to be continued until a residue is obtained which is completely soluble in 20 cc. distilled water. This last residue is supposed to consist of heparin.

The extreme dilution of the plasma used in this method was shown by Barratt to protect the antiprothrombin from injury by the high temperatures. Fuchs thinks that the high dilution probably brings about a dissociation of the prothrombin-antiprothrombin complex existing in the blood.

To test the validity of the method the procedure was carried through for

the blood of a peptonized dog, since it is known that the incoagulable blood obtained by this process contains a large excess of antiprothrombin (heparin). In a small dog a specimen of normal blood was obtained by cannula from the carotid in oxalate solution. Peptone solution was then injected into the femoral vein in the proportion of 0.4 gram of peptone per kilogram of animal. After twenty-five minutes another specimen of blood was obtained in oxalate solution. The process of peptonization was successful. A sample of the peptonized blood showed no signs of clotting after 24 hours.

The two specimens of oxalated plasma were put through the procedure described above and the residues finally obtained were examined for antiprothrombin as follows: Each residue was extracted with 2 cc. of 0.9 per cent solution of sodium chloride. The extract was filtered. Six thoroughly clean coagulation tubes were taken. In two of the tubes was placed the extract of the residue from the normal blood, 1 cc. in one tube and 0.5 cc. in the other. Two similar tubes were prepared with the extract of the residue from the peptonized blood and lastly two control tubes, one containing 1 cc. of 0.9 per cent solution of sodium chloride and the other 0.5 cc. A specimen of blood, 6 cc., was then taken by syringe from the arm vein of a normal individual; 1 cc. was emptied into each of the six coagulation tubes and the time of complete coagulation was noted. The following result was obtained:

1. Control tubes—clotting within 14 minutes.
2. Tubes with extract of residue from normal blood—one clotted in 35 minutes, the other in 50 minutes.
3. Tubes with extract of residue from peptonized blood—not clotted after 24 hours.

The results show that the method is valid for the detection of antiprothrombin in small quantities of blood. The normal blood yielded a distinct amount as shown by the prolongation of the clotting time compared with the control, while the peptonized blood contained sufficient to entirely prevent the clotting of 1 cc. of blood.

For the experiment upon hemophilic blood residues were prepared from the blood (5 cc. oxlated plasma) of three normal men and four specimens, taken on different days, of the blood of a hemophilic man, whose usual clotting time, for blood taken from the vein, lies between $2\frac{1}{2}$ and 3 hours. The extracts of the residues obtained from these specimens were compared, as regards their retarding effect on the clotting of blood, according to the schema outlined above. The results are given in the accompanying table.

TABLE 1

EXTRACT	COAGULATION TIME	CONTROL COAGULATION TIME
	minutes	*minutes*
Normal blood (E)	18.5	20
Hemophilic blood (J. Y.)	12.5	
Normal blood (D)	15.5	12
Hemophilic blood (J. Y.)	9.5	
Normal blood (O)	20.5	11.5
Hemophilic blood (J. Y.)	18.5	
Normal blood (E)	25	15
Hemophilic blood (J. Y.)	20	
Normal blood (E)	17.5	21
Hemophilic blood (J. Y.)	12	
Normal blood (E)	21	18.5
Hemophilic blood (J. Y.)	16	

CONCLUSION

The series is short but conclusive as regards the main point. No evidence at all was obtained of the presence of an excess amount of antiprothrombin in hemophilic blood. On the contrary the figures indicate just the reverse, that the hemophilic blood contains less rather than more antiprothrombin, as compared with normal blood.

The results by this method of Fuchs confirm therefore the previous findings obtained by Howell, using a different method, that involved relatively large amounts (100 cc.) of blood.

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THE "EXCESS RESPIRATORY QUOTIENT" OF THE RECOVERY PERIOD FOLLOWING STRENUOUS MUSCULAR EXERCISE IN MAN[1]

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There has been considerable discussion concerning the excess respiratory quotient of the recovery period following strenuous muscular exercise. Furusawa, Hill, Long and Lupton (1924) found values varying from 0.93 to 1.13 and concluded that the respiratory quotient of the excess metabolism caused by short-lived muscular effort of a severe nature appeared to be unity. At the same time, they realized that they were not dealing with complete recovery periods and the mean values obtained were slightly too high owing to too short a period of collection during recovery and thus only approximated unity. In an earlier paper Hill, Long and Lupton (1924c) reported values above unity but these values were dismissed on the basis that they were dealing with an incomplete recovery period. "These were among our earliest experiments, and the recovery period allowed was too short. The very large value of the ratio (excess CO_2)/(recovery O_2) shows that the recovery was certainly incomplete, and the oxygen debts, as recorded, too small." Furusawa (1925) obtained values varying from 0.98 to 1.05 for standing running at the rate of 64 to 244 steps per minute for short periods of time and allowing 10 to 40 minutes for recovery. Henderson and Haggard (1925) found that the respiratory quotient of exercise of a strenuous nature and recovery was approximately similar to that during rest. In these experiments only the gross respiratory quotients are given, their subjects were not basal before work and the recovery periods were not complete so no conclusions concerning the excess respiratory quotient can be drawn from their data. In a long series of experiments, Best, Furusawa and Ridout (1929) reported the respiratory quotient of very mild exercise as approximating the basal quotient, unity for moderate exercise, and values considerably above unity for strenuous work of short duration. Gemmill, Booth, Detrick and Schiebel (1931), during a study of the effect of training on the recovery period also obtained values above one, varying from 1.01 to 1.58 in one subject and 1.02 to 1.39

[1] A preliminary report of this work was made before the American Physiological Society, April, 1931, at Montreal, Canada.

in another subject with a one hour recovery period following running on a treadmill. In each experiment the oxygen consumption had not returned to normal at the end of the one hour recovery, the last sample being from ten to twenty cubic centimeters above the base line. The failure of the return of the oxygen consumption to the basal level within one hour after work has been observed by Hill, Long and Lupton (1924b) and Sargent (1926).

In order to see if complete experiments could be obtained within a reasonable length of time recovery periods of three or more hours were planned. If such an absolute experiment could be made with the carbon dioxide production and the oxygen consumption returning exactly to the basal base line, theoretically, a closer analysis of the excess respiratory quotient should be obtained. Small differences of ten or fifteen cubic centimeters between the basal determination and the values at the end of the recovery period when spread over a sufficient period of time will vary considerably the excess respiratory quotient.

METHOD OF EXPERIMENT. With this in view, we prolonged the recovery period to three or more hours. Before doing any work-experiments, basal control experiments were made over three hours to accustom the subject to breathing through a mouth piece for that period of time and to obtain the normal variation of the basal oxygen consumption and carbon dioxide production. The final procedure adopted was for the subject to sleep beside the treadmill[2] during the night preceding the experiment. In the morning an assistant took basal samples at ten minute intervals after a preliminary washing of the connections with expired air for twenty minutes. Two spirometers were connected by a valve so that by closing the inlet to one spirometer the expired air passed into the second spirometer. All the analyses were made in duplicate with the exception of the two basal control experiments on C. G. on October 7th and November 12th. A standard Haldane gas analyzer of ten cubic centimeter capacity was used. The deviations of the analyses made in duplicate are plotted against the percentage occurrence of each deviation in 480 analyses in figure 1. This figure illustrates that the majority of duplicate analyses agreed within 0.04 per cent.

The results of the four basal experiments on C. G. are given in graphic form in figure 2. The variation between samples becomes less as the subject becomes more accustomed to the experimental procedure, being practically linear in the basal experiment of November 19th.

After the preliminary basal determinations work-experiments were undertaken. As in the case of the basal determinations the subject came to the laboratory the night before the experiment, slept in a bed beside the

[2] The author wishes to thank the Helen Hartley Jenkins Fund for Medical Research for financial aid in obtaining the treadmill.

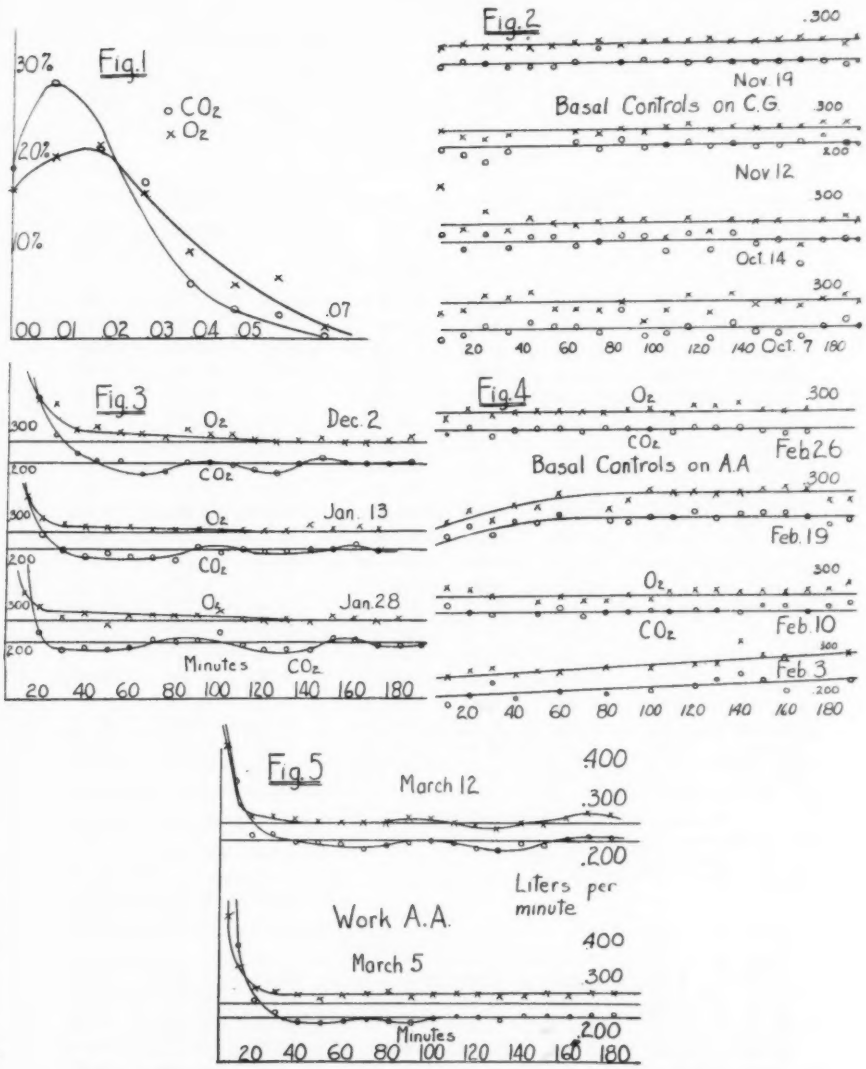

Fig. 1. Deviation between duplicate analyses in terms of CO_2 and O_2 percentages plotted against the per cent of the total number of analyses.

Fig. 2. Basal controls of C. G.

Fig. 3. Recovery periods after running of C. G.

Fig. 4. Basal controls of A. A.

Fig. 5. Recovery periods after running of A. A.

TABLE 1

BASAL. NOVEMBER 19, 1930				WORK. JANUARY 28, 1931			
Time	Ventilation, 0° and 760 mm.	O_2	R.Q. gross	Time	Ventilation, 0° and 760 mm.	O_2	R.Q. gross
Subject C. G.							
minutes	*liters per minute*	*liters per minute*		*minutes*	*liters per minute*	*liters per minute*	
				Basal	5.55	0.273	0.84
				Recovery			
0–10	5.64	0.254	0.83	0.4–2.0	42.47	1.796	1.04
10–20	5.73	0.262	0.87	2.0–5.5	18.18	0.515	1.46
20–30	6.03	0.265	0.83	5.5–11.00	10.60	0.343	1.16
30–40	5.56	0.252	0.85	11–20	6.82	0.303	0.83
40–50	5.58	0.254	0.84	20–30	5.64	0.281	0.76
50–60	5.54	0.257	0.82	30–40	5.84	0.292	0.75
60–70	5.58	0.264	0.85	40–50	5.72	0.273	0.78
70–80	6.14	0.268	0.94	50–60	5.46	0.286	0.77
80–90	5.41	0.258	0.86	60–70	5.88	0.288	0.82
90–100	5.56	0.266	0.86	70–80	5.89	0.286	0.81
100–110	5.79	0.267	0.84	80–90	5.71	0.287	0.81
110–120	5.88	0.266	0.82	90–100	6.13	0.298	0.84
120–130	5.79	0.269	0.84	100–110	5.57	0.276	0.81
130–140	5.68	0.265	0.83	110–120	5.34	0.275	0.78
140–150	5.58	0.261	0.84	120–130	5.94	0.279	0.76
150–160	5.55	0.262	0.84	130–140	5.63	0.271	0.78
160–170	5.79	0.269	0.83	140–150	6.07	0.286	0.83
170–180	5.80	0.265	0.82	150–160	6.04	0.280	0.85
180–190	5.58	0.252	0.82	160–170	5.80	0.273	0.82
190–200	5.80	0.266	0.81	170–180	5.70	0.280	0.80
200–210	5.79	0.262	0.84	180–190	5.83	0.274	0.80
Subject A. A.							
BASAL. FEBRUARY 26, 1931				WORK. MARCH 12, 1931			
				Basal	4.92	0.230	0.83
					5.03	0.236	0.82
				Recovery			
0–10	4.93	0.228	0.84	0.21–2.00	32.21	1.263	1.16
10–20	5.06	0.249	0.85	2–5	17.08	0.403	1.48
20–30	4.77	0.234	0.80	5–10	10.00	0.301	1.10
30–40	5.06	0.239	0.84	10–18	5.93	0.249	0.83
40–50	5.09	0.242	0.84	18–28	5.91	0.250	0.84
50–60	5.07	0.242	0.84	28–38	5.32	0.244	0.79
60–70	4.79	0.241	0.82	38–48	5.29	0.238	0.80
70–80	5.17	0.238	0.85	48–58	5.11	0.237	0.78
80–90	5.22	0.246	0.82	58–68	4.56	0.233	0.75
90–100	5.12	0.248	0.81	68–79	4.81	0.232	0.80

TABLE 1—*Concluded*

BASAL. FEBRUARY 26, 1931				WORK. MARCH 12, 1931			
Time	Ventilation, 0° and 760 mm.	O_2	R.Q. gross	Time	Ventilation, 0° and 760 mm.	O_2	R.Q. gross
Subject A. A.—*Concluded*							
minutes	*liters per minute*	*liters per minute*		*minutes*	*liters per minute*	*liters per minute*	
100–110	5.03	0.236	0.82	79–88	4.85	0.248	0.76
110–120	5.19	0.257	0.80	88–98	5.00	0.245	0.78
120–130	5.18	0.253	0.80	98–109	4.94	0.232	0.81
130–140	5.15	0.260	0.79	109–118	4.56	0.226	0.79
140–150	4.99	0.244	0.80	118–128	4.77	0.218	0.81
150–160	4.86	0.240	0.79	128–138	4.99	0.233	0.81
160–170	4.87	0.237	0.81	138–148	4.93	0.228	0.81
				148–158	5.25	0.248	0.80
				158–168	5.42	0.258	0.80
				168–178	5.17	0.255	0.79

treadmill, in the morning after a preliminary breathing period of twenty minutes to wash out connections and to allow any slight alteration of the breathing to return to normal a basal sample of expired air was collected for ten minutes, in some cases, two samples were taken. The subject then put on a track suit, climbed on the treadmill, ran sixty-five to seventy yards in ten seconds holding his breath, returned to the bed and a continuous recovery period of three hours was obtained.

After doing three work-experiments on C. G. similar experiments were made on A. A. Four basal control experiments were made and the results are given graphically in figure 4. A. A. never had acted as a subject for metabolic experiments and his basal control experiments showed greater variations than those of C. G. Two work experiments were made on A. A.

The individual data for the two subjects are as follows: C. G., age 29, height 180 cm., weight 82 kilograms. A. A., age 18, height 175 cm., weight 61 kilograms.

RESULTS. The results of the three work-experiments on C. G. are given graphically in figure 3 and for the two experiments on A. A. in figure 5. The numerical results of the basal control experiment on C. G. of November 19th, the work-experiment of January 28th, the basal control on A. A. of February 26th and his work-experiment of March 12th are given in table 1. It may be observed that the oxygen consumption returns to the base line within two hours in all the experiments on C. G. and in the second experiment on A. A. In the first experiment on A. A. the oxygen consumption returns to a value that parallels and is twenty cubic centimeters above the basal value. The carbon dioxide production shows rhythmic changes

during the recovery period, not being within the normal variation until the end of the three-hour period in the majority of the experiments.

A summary of all the results is given in table 2. In the first experiment on C. G. the oxygen debt was 7.78 liters with an excess respiratory quotient of 1.1, the other two experiments gave excess respiratory quotients similar to the basal quotients. In A. A. the experiment with a complete recovery gave an excess respiratory quotient of 1.0. There is definite carbon dioxide retention in all the experiments varying from 0.757 liter to 1.315 liters. The importance of obtaining a complete experiment is illustrated by the excess respiratory quotients calculated at the end of one hour recovery, all of which are above 1.0 and from the first experiment on A. A. where the final value at the end of the three hour recovery period is twenty cubic centimeters above the basal value. In this experiment we have the choice of three base lines, the basal value, the average of the basal and the final values and the final value. If we take the basal value for the base line the

TABLE 2

	SUBJECT C. G.			SUBJECT A. A.
	December 2, 1930	January 13, 1931	January 28, 1931	March 12, 1931
Basal R.Q.	0.81	0.88	0.84	0.82
Gross R.Q.	0.85	0.87	0.84	0.84
1 hour excess R.Q.	1.31	1.46	1.14	1.38
2 hours excess R.Q.	1.17	1.21	1.10	1.15
3 hours excess R.Q.	1.10	0.86	0.85	1.00
Oxygen debt, liters	7.78	5.52	5.25	3.73
CO_2 retention, liters	0.757	1.172	1.315	1.184

excess quotient is 0.83; taking the average of the basal and the final values as Best, Furusawa and Ridout (1929) did in their experiments we get an excess respiratory quotient of 1.13. If we take the final value, a method used by Hill, Long and Lupton (1924c) we obtain an excess R.Q. of 1.77. It is very apparent that consistent results depend on the final values agreeing very closely with the basal values. This is the explanation of the high values of the excess respiratory quotients obtained by Best, Furusawa and Ridout (1929) and by Gemmill, Booth, Detrick and Schiebel (1931), as the recovery period was never complete in the time allowed for the determinations. In the work of Best and his associates (1929) on mild and moderate exercise, the oxygen, carbon dioxide and respiratory quotient values for the first resting and final resting samples practically agree, but in many experiments following severe exercise, these values show deviations. For example, in table V of their paper, the deviations for the two values of oxygen consumption vary from -1 cc. to $+21$ cc. and of carbon

dioxide production from −24 cc. to +35 cc. with corresponding deviations between the respiratory quotients. The deviations in table IV vary for O_2 from −5 to +13 and for CO_2 from −7 to +17.

Discussion of results. Has the term excess respiratory quotient and excess metabolism any exact physiological meaning? This question has been discussed by the author and his associates (Gemmill, Booth and Pocock, 1930; Gemmill, Booth, Detrick and Schiebel, 1931) in connection with the problem of the base line for work experiments and it was concluded that the excess metabolism is not a satisfactory measure for comparative experiments. The same conclusion is now reached for the term "excess respiratory quotient." Theoretically, this term gives an indication of the type of metabolism that is taking place during muscular work. Practically, there are many difficulties in obtaining such a quotient, especially for long recovery periods. Being an excess quotient, it involves four values each with a plus and minus deviation. An attempt must be made to fit a curve with a plus and minus deviation into a base line, the base line also having a plus and minus deviation, in order to obtain the point where recovery is complete. This involves a difficult mathematical analysis. The time factor is important, as is shown from the calculation of the excess respiratory quotients for the end of the first, second and third hour of the recovery periods in table 2. The excess respiratory quotient shows a decrease as the recovery becomes more complete, but it is practically impossible to tell when it is absolutely complete.

The base line in all cases is assumed to be a constant. That is probably not a correct assumption for it is very likely that the base line shifts during and after exercise. A slight deviation in the base line will affect tremendously the excess respiratory quotient. Assume, for example, a complete three hour recovery of sixty liters of oxygen with a gross respiratory quotient of 0.8 and a basal value of fifty liters with the same respiratory quotient. The excess quotient in this case would be 0.8. Now change the basal value to fifty-one liters, a two per cent deviation, the basal quotient would be 0.78 while the excess quotient would be 0.88. Thus a two per cent deviation in the base line would change the excess quotient from 0.8 to 0.88. The deviations in any metabolic determination are much greater. The maximal deviation in the best basal determinations on C. G. was 7 per cent for the oxygen values and 20 per cent for the carbon dioxide values. On A. A. a deviation of 14 per cent was obtained for his oxygen consumption and 10 per cent for his carbon dioxide output. We have no method of determining the percentage deviation in the determinations after work without a very large series of experiments. It is very probable that these deviations are larger than those for basal determinations as we would be dealing with a more complex system during work. A ten per cent deviation in the basal oxygen consumption would change the excess quotient from 0.8 to 1.6.

Taking into consideration the above facts, one is forced to the conclusion that the excess respiratory quotient is a mathematical abstraction that has no physiological basis which can be determined experimentally with our present metabolic methods.

Another factor may be the conversion of fat into carbohydrate during and after exercise. If ever this conversion is proven definitely to occur, the respiratory quotient as an exact index of specific metabolism will have lost all meaning.

In four out of five work experiments the oxygen consumption came back to the base line within two hours after work. Hill, Long and Lupton (1924b) and Sargent (1926) did not obtain complete recovery periods, both series of experiments giving final values averaging seven per cent above the basal samples. They concluded that there was a small but definite change in the basal metabolism and this change was not associated with the recovery period. The results obtained in this series of experiments show that it is possible with adequate basal precautions and a two hour recovery period to obtain a complete recovery in the oxygen consumption. Thus the work produces no change in the demand for oxygen which is not returned completely to the previous basal state during the recovery period.

The slow return to normal of the metabolism after work has been described by many observers, the earlier literature being discussed by Benedict and Cathcart (1913, p. 163). From their own experiments, Benedict and Cathcart concluded that the influence of exercise may persist for five or six hours after work has stopped.

The logistic function fits the data for the return of the oxygen consumption to normal following exercise as in previous work (Gemmill, Booth, Detrick and Schiebel, 1931).

A complete return of the carbon dioxide to normal following work was also obtained. In some of the experiments two periods of carbon dioxide retention are observed, one coming in the first hour and the other at the end of the second hour. This was not so marked in the experiment of January 13th on C. G. and the experiment of March 5th on A. A., but is definite in the other three experiments. In the experiments where it is present, the curve is similar to the swinging back to normal of a heavily dampened mechanical system, finally swinging back within normal limits towards the end of the third hour. No adequate explanation of the phenomenon can be given at the present time. It may be an over and under compensation of the mechanisms for regulating carbon dioxide tensions but further study is necessary before this curve may be analyzed.

A number of observers working on the respiratory quotients of the recovery period have placed emphasis on the return of the oxygen consumption back to the normal value as evidence of complete recovery.

Since the respiratory quotient is a quotient of two variables, just as much emphasis should be placed on the return of the carbon dioxide back to normal which in our experiments comes after the complete oxygen return.

Taking the individual periods after work, the respiratory quotient showed a primary rise above one, a gradual fall below the basal value, a return to normal and in some cases followed by a second fall and rise. The early changes have been observed by Krogh and Lindhard (1920) and Campbell, Douglas and Hobson (1921) (these observers giving references to earlier work on this subject), and by Hill and his associates (1924a). The second period of carbon dioxide retention seen in three of our experiments has not been described previously by these observers.

SUMMARY

A study of the recovery period following strenuous exercise of short duration was made on two individuals.

Satisfactory basal controls were obtained over three hour periods after repeated trials.

The oxygen returned to the base line within two hours in four experiments. In one experiment it remained parallel and twenty cubic centimeters above the base line.

The carbon dioxide production returned to the base line within three hours. In three experiments there were two major periods of carbon dioxide retention.

A consideration of the inherent and unavoidable errors in the calculation of the "excess respiratory quotient" shows that the excess respiratory quotient for long recovery periods is a mathematical abstraction. It cannot be evaluated experimentally with our present methods with any degree of precision and thus is useless from the standpoint of determining the type of metabolism characteristic for exercise.

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STUDIES ON THE ADRENAL CORTEX

IV. Further Observations on the Preparation and Chemical Properties of the Cortical Hormone[1,2]

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In previous communications (Anat. Record, 1929, xliv, 225; Science, 1930, lxxi, 321; lxxi, 489; lxxii, 75; lxxii, 482; this Journal, 1931, xcvi; Journ. Amer. Med. Assoc., 1931, xcvi) the writers described a suitable method for extracting the cortical hormone from fresh beef adrenal glands. The effect of administration of the extract upon animals suffering from adrenal insufficiency and human patients with Addison's disease has been studied in detail. This paper is concerned with presentation of additional data regarding the preparation and chemical properties of the cortical hormone.

The criterion employed for testing the potency of various fractions was similar to that outlined in study III (This Journal, 1931). This criterion was the revival of cats prostrate from adrenal insufficiency, and restoration of the animals to an apparently normal condition following subcutaneous or intraperitoneal injections of extract. Following disappearance of symptoms and return to normal activity, the extract treatment was discontinued and the cats permitted to redevelop symptoms and die of adrenal insufficiency.

In testing various preparations for potency, each sample used was compared with fraction B of the type of extract described in detail in study III (This Journal, 1931). Fraction B will restore animals prostrate from adrenal insufficiency and on the verge of death to normal condition within 72 hours or less when used either subcutaneously or intraperitoneally. This fraction served as a rough qualitative standard for comparison of other types of extract. Some of the preparations tested and discarded as giving essentially negative results, in reality probably contained small amounts of cortical hormone, but the quantity was very small as compared with that contained in our standard fraction B material.

[1] A preliminary report of this work was presented to the Society for Experimental Biology and Medicine, February 18, 1931; see Proceedings, xxviii, p. 510, 1931.

[2] This investigation has been aided by a grant from the Josiah Macy Jr. Foundation of New York.

In preparing physiologically potent material for the further fractionation studies reported in this communication, the scheme of fractionation previously reported in detail (study II, This Journal, 1931) was used with one simplification. The concentration of the petroleum-ether phase and washings after the first distribution (of the acetone-soluble fraction between petroleum-ether and 70 per cent alcohol) is omitted and instead the second distribution is executed directly on the petroleum-ether phase resulting from the first distribution.

Fractionation with permutit[3] has made possible the preparation of active extracts from whole beef adrenal glands thereby doing away with the expense of dissection. Potent extracts were prepared by simply extracting whole adrenal glands (freed from extraneous fat and connective tissue) by the process previously published in detail for the preparation of extracts from dissected cortex (studies II and III, This Journal, 1931). Each cubic centimeter of whole gland extract represents 50 grams of whole adrenal glands. The adrenalin concentration of the finished extracts varies between 1:1,250,000 and 1:2,500,000 while the solid content ranges from 0.3 to 0.4 per cent with the exception of one preparation in which the solid content was 0.47 per cent. The following two sample protocols taken from the several animals studied in this connection, show quite clearly that extracts made from whole adrenal glands are active and will restore to apparently normal condition cats prostrate from adrenal insufficiency. Subcutaneous administration is just as effective as intraperitoneal use. Whole gland extract can also be used intravenously. The adrenalin assays of three extracts prepared from whole glands together with other data which are self explanatory are given in table 1.

Protocol W. G. 1. Adult male. Weight 2700 grams. Right gland removed September 1, 1930. Left gland removed September 6. Weight 2700 grams. September 6–11, no symptoms. September 11, first symptoms noted. September 12, 8 p.m., cat exhibited severe symptoms. Rectal temperature 94°, animal staggers about when placed upon its feet, given 7 cc. extract intraperitoneally at 9 p.m. Weight 2550 grams. September 13, animal about the same as previous day. Rectal temperature 96°. Given two injections of 7 cc. each. September 14, cat bright and lively, eats liver eagerly, walks about in a normal manner. Rectal temperature 100.2°. Given two 7 cc. injections during day. September 15, cat eats liver and drinks milk. Rectal temperature 101.6°, no symptoms. Extract discontinued. Weight 2746 grams. September 16–19, cat normal. September 19, symptoms again appeared. September 20, cat prostrate. Animal died at 8 p.m. Weight 2541 grams.

Protocol W. G. 2. Adult male. Weight 3140 grams. Right adrenal removed August 27, 1930. Left adrenal removed September 4; weight 3167 grams. September

[3] Prepared by The Permutit Company, 440 Fifth Ave., New York City, for urinary ammonia determination according to Folin. It is reclaimed by rinsing in order with hot 5 per cent sodium hydroxide, tap water, 2 per cent acetic acid, distilled water, and a small quantity of 95 per cent alcohol, followed by drying in an oven.

5–14, cat normal. September 15, slight symptoms apparent, such as anorexia and weakness in hind legs. Rectal temperature 97°. Weight 3013 grams. September 16, marked symptoms. Animal staggers about when walking. Lies prostrate most of the time. Rectal temperature 94°. Injected with 8 cc. extract intraperitoneally twice daily. Weight 2950 grams. September 17, cat about the same. Extremely weak. Injected twice daily same as on previous day. Rectal temperature 94°. September 18, animal much improved. Walks about. Eats bits of liver and drinks milk. Rectal temperature 98°. Injected as before. September 19, cat normal except for slight weakness in hind legs when walking. Eats heartily. Rectal temperature 101.2°. Injected as before. September 20, cat bright and lively. No symptoms whatever. Eats eagerly and runs about. Injections discontinued. September 21–24, cat remained in normal condition. Weight on September 24 was 3200 grams. September 25, symptoms reappeared and animal died September 26 presenting typical symptoms of adrenal insufficiency.

TABLE 1

Whole adrenal gland extract (permutit fractionation)

PREPARATION NUMBER	WEIGHT OF FRESH GLANDS	WEIGHT OF ACTIVE FRACTION	ADRENALIN IN ACTIVE FRACTION (BIO-ASSAY: BLOOD PRESSURE)	ACTIVE FRACTION PER KILO FRESH GLANDS
	grams	*grams*	*mgm.*	*mgm.*
150-P1	4,950	0.36	0.040	73
150-P4	4,855	0.34	0.078	70
150-P5	4,895	0.47	0.049	96

TABLE 2

Comparison of cortex and whole gland extraction (averages of 5 preparations)

FRACTION	DISSECTED CORTEX	WHOLE GLANDS
	grams per kilo	*grams per kilo*
Benzene-soluble	28.2	25.8
Acetone-soluble	7.6	6.3
70 per cent alcohol-soluble	0.56	0.80
Permutit-purified	0.150	0.106
Final water-soluble	0.087	0.072

So far as our data go they indicate that whole gland extract is as effective in combatting adrenal insufficiency as are preparations of cortex alone. In preparing extracts on a small research scale, however, we have found it more economical from both the standpoint of time and cost of solvents, to work with dissected material. From the standpoint of chemical purification it is also advantageous to work with dissected material as the data in table 2 would indicate. Since cortex comprises about two-thirds of the weight of the adrenal gland, it is to be expected that one kilogram of whole gland would yield about two-thirds of the activity obtainable from one kilogram of dissected cortex.

In order to render the extract (prepared either from adrenal cortex or whole adrenal gland) suitable for intravenous injection, it was necessary to remove the water-insoluble fraction after permutit purification (study III, This Journal, 1931). This was accomplished by Sietz filtration. The following experiments indicate that a small fraction of the activity may be lost at this point.

The water-insoluble fraction from a batch of 6645 grams of adrenal cortex was suspended in 150 cc. of water and used in the following experiment:

Protocol W. I. 1. Adult male. Right gland removed October 28, 1930. Left gland removed November 6. Weight 2941 grams. November 7–14, animal normal. November 14, first symptoms noted. November 16, pronounced symptoms. Animal prostrate. Weight 2755 grams. Injected 6 cc. of extract subcutaneously four times daily. November 17, condition same as before. Injected same as on previous day. Ate liver at 4 p.m. November 18, animal looks bright, has excellent appetite but is weak. Twenty cubic centimeters extract given in four doses. November 19, animal shows weakness when walking but eats fairly well. Rectal temperature 100°. Given 4 injections of 6 cc. each. November 20, cat same as before. Four 6 cc. injections. November 21, cat very weak. Given one 6 cc. injection, animal died at 12:30 p.m. Weight 2490 grams.

Cat W. I. 1, although it received the water-insoluble fraction from 5400 grams of adrenal cortex died with typical symptoms of adrenal insufficiency. The water-soluble fraction obtained from this quantity of material was sufficient to restore several cats prostrate from adrenal insufficiency to a normal condition. The slight improvement observed in this animal prompted us to test this point further.

The water-insoluble fraction obtained from 79 kilograms of adrenal cortex (this fraction weighed 3.954 grams) was suspended in 200 cc. of water and used in treating the following two animals:

Protocol W. I. 2. Adult male. Right gland removed October 25, 1930. Left gland removed November 15. Animal used for testing other active fractions until December 13; on this date, cat in excellent condition and eating heartily. December 20, slight symptoms apparent. Refuses food. December 22, distinct symptoms present. Weight 2775 grams. December 23, cat verging on prostration, can barely walk; given 6 cc. four times daily subcutaneously. December 24, cat same as before. Given four 6 cc. injections. December 25, cat shows great improvement. Walks about. Seems lively. Injected as before. December 26, animal drinks milk and eats liver eagerly. Given but one 6 cc. injection. Treatment discontinued on this date. December 27, cat in good condition. Drinks milk. December 28, refused all food. December 29, marked symptoms developed. Animal near prostration. Used for testing a different fraction on this date. Animal later died of adrenal insufficiency.

Protocol W. I. 3. Adult female. Right adrenal removed November 15, 1930. Left gland removed November 22; weight 3000 grams. This animal used for testing other fractions until December 5, when it was in excellent health. December 3–7, cat

normal. December 8, refuses all food. Weakness in legs apparent. December 9, marked symptoms. Cat staggers about when walking. Injected 6 cc. extract four times daily. December 10, cat greatly improved. Eats heartily, runs about in normal fashion. No symptoms. Injections continued. December 11, cat normal. Injections discontinued this date. December 12–18, cat normal. No symptoms. December 20, first symptoms. December 23, cat died with typical symptoms of adrenal insufficiency.

The positive evidence presented in protocols W. I. 2 and W. I. 3 shows clearly that a small amount of activity remains in the water-insoluble fraction. In the routine preparation of extract this activity can be readily reclaimed by extracting the water-insoluble fraction from the Seitz pad (used in clarifying the extract) with alcohol and proceeding as in the usual final clarification step (study III, This Journal, 1931, p. 183).

One of the most difficult problems which has arisen in the course of the work has been the separation of adrenalin from the active fractions of the cortex without appreciably diminishing the yield of cortical hormone.

TABLE 3

Adrenal cortex extract (aqueous alkali-ether fractionation)

PREPARATION NUMBER	WEIGHT OF CORTEX	WEIGHT OF ACTIVE FRACTION	ADRENALIN IN ACTIVE FRACTION (BIO-ASSAY: BLOOD PRESSURE)	ACTIVE FRACTION PER KILO FRESH CORTEX
	grams	*grams*	*mgm.*	*mgm.*
153-P1	3,175	0.286	0.026	90
153-P2	3,340	0.255	0.028	76

Recently another method, aside from permutit fractionation has been devised for successfully accomplishing this step. It consists in distributing an active fraction between aqueous alkali and an immiscible solvent such as benzene or ether. Adrenalin passes into solution in the aqueous alkali whereas the cortical hormone is found in the immiscible solvent phase. By means of this fractionation step, highly active extracts (1 cc. equivalent to 30 grams cortex) have been prepared containing less than one part of adrenalin in 4,000,000. The data on two typical experiments are recorded in table 3. This type of extract can be used subcutaneously, intraperitoneally or intravenously.

The details of this fractionation step are best illustrated by citing the following experiment:

The 70 per cent alcohol-soluble fraction obtained from 3 kilograms of fresh beef cortex served as the starting material. This solution contained 1.58 grams of solids including 27 mgm. of adrenalin.

The solvents are removed by distillation in partial *vacuo* at an external temperature of 45–50°C. Toward the end of the distillation small quantities (about 30 to 50 cc.) of absolute ethyl alcohol are added to facilitate

the removal of water. The residue is transferred to a separatory funnel with 100 cc. of ether. The ether solution is washed with five 50 cc. portions of 0.1 N sodium hydroxide, and then with three 50 cc. washings of distilled water. The alkali and water washings are washed in order with one 100 cc. portion of fresh ether. The ether solution and ether washing are combined, the ether removed by distillation in the usual manner and the residue taken up in 100 cc. of 95 per cent alcohol.

Eighty cubic centimeters of water are added, the alcohol removed by distillation as usual, the volume brought to 100 cc. with water and the extract clarified by Seitz filtration. The finished extract contained 0.23 gram of solids. It is a clear pale yellow solution suitable for subcutaneous, intraperitoneal or intravenous use. It is rendered isotonic by the addition of 0.8 per cent sodium chloride.

The activity of this type of extract is demonstrated in the two typical protocols which follow:

Protocol A. W. 1. Adult female. Right adrenal removed November 15, 1930. Left gland removed November 22, weight 3000 grams. November 22–27, animal normal. November 28, cat exhibiting symptoms such as anorexia, weakness in legs when walking, falling rectal temperature. Injected twice during day subcutaneously with 6 cc. of extract. November 29, cat same as before. Injected four times daily with 6 cc. extract. November 30, cat ate salmon and seems greatly improved; 6 p.m., animal killed and ate white rat placed in her cage. December 1, animal crying for food. No symptoms, ate liver voraciously. Weight 2850 grams. Extract discontinued on this date. December 2–8, animal remained normal. December 9, marked symptoms appeared. Animal used for testing other fractions. Died later of adrenal insufficiency.

Protocol A. W. 2. Adult male. Right adrenal removed October 25. Left adrenal removed November 25. Weight 3500 grams. This animal used for testing other fractions until December 26. Cat in excellent condition at this time. December 27–28, no symptoms. December 29, very marked symptoms present. Animal verging on prostration. Injected 6 cc. of extract subcutaneously, two times daily. Weight 2600 grams. December 30, injected 6 cc. four times daily. Animal drank milk and ate kidney. December 31, very great improvement. Animal runs about and seems lively. Eats heartily. Animal very hungry. January 1, animal in excellent condition. Dosage reduced to 2 cc. 3 times daily. January 3, extract discontinued. Cat in perfect condition. Weight 2715 grams. January 7, animal in perfect condition. Weight 2785 grams. January 12, symptoms again appeared and the animal was used for testing other fractions. Died later of adrenal insufficiency.

An attempt to separate adrenalin from the cortical hormone by distributing an active fraction between an immiscible solvent and aqueous acid met with failure. The type of fractionation procedure outlined above was used on several different batches of material except that 0.1 N hydrochloric acid was used instead of 0.1 N sodium hydroxide. The adrenalin present in the active fraction is removed in the acid washings

but our results to date indicate that the distribution of the cortical hormone is such that this fractionation step cannot be used to advantage.

Although the cortical hormone can be separated from adrenalin by means of dilute aqueous alkali, it is apparently destroyed by saponification. The 70 per cent alcohol-soluble fraction obtained from 3160 grams of adrenal cortex was dissolved in 75 cc. of absolute alcohol. Twenty-five cubic centimeters of normal sodium ethylate were added and the mixture kept at room temperature for one hour with frequent agitation. The alcohol was removed under reduced pressure at 40°C. and the residue thoroughly extracted with ether. The ether solution was washed three times with 50 cc. portions of distilled water. The ether was removed as usual, the residue taken up in alcohol, transferred to 105 cc. of water, passed through a Seitz filter and the filtrate tested on bilaterally adrenalectomized cats showing marked symptoms. This extract was totally inactive.

The cortical hormone is thermolabile. Highly potent cortical extracts are rendered inactive by boiling gently in an open flask for two minutes.

Extract of beef adrenal cortex prepared with permutit fractionation gives a negative biuret, ninhydrin, Hopkins-Cole, Molisch, Pauly,[4] Knoop (Hunter's modification), and Liebermann-Burchard reaction. It gives a positive xanthoproteic, Millon's, alkaline copper, and alkaline phosphotungstate reaction. These four positive reactions can be accounted for by the presence of traces of phenolic decomposition products of adrenalin.

In previous communications we have stated that the finished unpreserved extract retains its potency apparently undiminished for several weeks when kept at about 6°C. We have collected data showing that the extract retains its activity for comparatively long periods when kept at room temperature if preserved with 0.1 per cent benzoic acid. Preserved extract stored for 45 days at room temperature during the months of July, August and the first week in September was found to have retained its original potency when tested upon adrenalectomized cats. Protocols of two of the animals are given below. The data indicate clearly that benzoic acid is an excellent preservative when added to extracts containing the cortical hormone. It is unfortunate that our experiments could not have extended over longer periods but to date we have not been able to make sufficient extract to permit us to put aside any considerable amount for extensive study of its keeping qualities under various conditions.

Chloretone, when added to extracts containing the cortical hormone does not exert any preservative action. We have tested such material

[4] This test was called negative because no red or pink color developed. A brownish-yellow color developed which could possibly have masked a faint pink. The development of this interfering color may be due to the presence of traces of phenolic decomposition products of adrenalin in the extract.

after it had been left at room temperature for several weeks and found it inactive.

Protocol B. P. 1. Adult male. Right gland removed July 22. Left gland removed July 31. This animal was used for testing another fraction until August 9, 1930. On this date the animal showed slight symptoms such as anorexia and hind leg weakness. It was injected twice daily with 1.5 cc. of extract preserved with 0.1 per cent benzoic acid. Weight 2741 grams. August 10–12, all symptoms disappeared. August 12 to September 4, cat remained in perfect health and gained weight. Weight this date 2901 grams. The benzoic acid preserved extract injections were discontinued September 5. Animal developed severe symptoms of adrenal insufficiency and was again used for testing other fractions.

Protocol B. P. 2. Adult male. Right gland removed July 12. Left gland removed August 11, 1930. Weight 2320 grams. August 12, animal injected twice daily with 1.5 cc. of benzoic acid preserved extract. August 13 to September 4, cat remained in excellent condition, each day receiving two injections of 1.5 cc. Weight 2640 grams. September 5, extract discontinued. September 15, symptoms adrenal insufficiency developed. Cat died of adrenal insufficiency.

Further tests of the keeping quality of the hormone in benzene have been made and the results support our earlier statements that the hormone can be safely stored in this solvent. The benzene soluble fraction was prepared in the usual way from 2700 grams of adrenal cortex received in the laboratory on November 26, 1929. The tissue was stored in alcohol from November 26 to December 20. The benzene soluble fraction was stored in benzene at 8°C. until February 10, 1931 when it was fractionated by the methods previously described (studies II and III, This Journal, 1931). The finished extract which essayed less than 1:2,000,000 adrenalin was active as is evidenced by the following protocol:

Procotol K. G. 1. Adult female. Right adrenal removed February 16. Left gland removed February 23, weight 3000 grams. February 24–27, no symptoms. February 28, slight symptoms adrenal insufficiency. March 1, exhibits marked symptoms, animal walks with difficulty, verging on prostration. Injected 5 cc. extract subcutaneously at 6:30 p.m. March 2, cat in bad condition, can barely stand erect when placed on its feet. Injected 20 cc. in four doses during day. March 4, cat greatly improved, walks about without symptoms, ate small amount of liver at 4 p.m., drank some milk. Injected 20 cc. in four divided doses during day. March 5, cat shows no symptoms, injected 5 cc. during day. Extract discontinued this date. March 9, cat again developed severe symptoms of adrenal insufficiency and died on March 10.

A similar result was obtained with a fraction stored from February 27, 1930 to March 28, 1931. The nature of the criterion used does not give a strict quantitative concept of the hormone content of these fractions but it does supply conclusive evidence that much of the activity is retained by active fractions stored in this manner for periods up to twelve to fifteen months. Extracts prepared from fractions stored for several months

have a light red color in contrast with the light yellow color of extracts prepared without delay, although there is no appreciable difference in solid content. The red color is due to the presence of oxidation products of adrenalin and adrenalin-like substances.

SUMMARY AND CONCLUSIONS

1. Extract made from whole adrenal glands is as effective in combatting adrenal insufficiency and as low in adrenalin content as are preparations of cortex alone.

2. A new method for separation of adrenalin from the cortical hormone is described. It consists in distributing an active fraction between aqueous alkali and an immiscible solvent such as benzene or ether. Adrenalin passes into solution in the aqueous alkali whereas the cortical hormone is found in the immiscible solvent phase.

3. By means of the above fractionation step, highly active extracts (1 cc. equivalent to 30 grams of cortex) have been prepared, containing less than one part of adrenalin in 4,000,000.

4. The cortical hormone is thermolabile and is destroyed by boiling an active extract in an open flask for two minutes.

5. Cortical extracts prepared with permutit fractionation give a negative biuret, ninhydrin, Hopkins-Cole, Molisch, Pauly and Liebermann-Burchard reaction. They give a positive xanthoproteic, Millon's, alkaline copper, and alkaline phosphotungstate reaction. These four positive reactions can be accounted for by the presence of traces of phenolic decomposition products of adrenalin.

6. Cortical extracts, if preserved with 0.1 per cent benzoic acid, retain their potency for long periods at room temperature.

7. Tests of the potency of fractions containing the cortical hormone which had been stored in benzene for 12 to 15 months, show that they retained much of their activity during this period although they are apparently somewhat less potent than freshly prepared material.

ACTIVITY OF THE ISOLATED UTERUS AND ITS RELATION TO THE OESTROUS CYCLE IN THE ALBINO RAT

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A number of workers (1), (2), (3), (4), (5), (6), reporting on the spontaneous rhythm of the excised uterus, have as a rule found less activity at oestrus with some irregularity in rate and extent of contraction. The present study was made to ascertain whether there is any definite relation between the stage of oestrus as determined by the smear method of Long and Evans (7) and the rate of contraction and whether there is a characteristic difference in the rate of the two ends of the uterine horn. Thirty mature virgin females quite definitely in oestrus or dioestrus were used in the experiments.

The animals were put under chloroform, the abdomen opened, the horns freed from the mesentery and excised by transection through Fallopian tube and cervix. One horn was then bisected transversely, the two segments suspended in aerated Tyrode's solution and connected to Becker levers. The leverage, distance from the muscle, and the weight carried were the same in all cases. After waiting for 15 minutes for the muscles to recover their tone, the contractions were recorded on Harvard drums making one rotation per hour. (Figs. 1 and 2.)

As seen in the graphs the movements of the isolated uterus were fairly regular in frequency and amplitude, although both varied to a slight degree in different animals and sometimes in the same animal. Following Blair's method (1), (2), consideration was given only to major or near major contractions apparently involving all or nearly all of the muscular tissue. There appeared to be no significant relation between amplitude and the different stages of the oestrous cycle.

The rates of the rhythmic contractions from different animals are given below.

Rate per hour in dioestrus:
Ovarian end: 63, 53, 66, 46, 47, 56, 54, 58, 32, 28, 69, 49, 55, 37, 35, 43. Average 48.
Vaginal end: 48, 45, 45, 36, 39, 40, 46, 38, 18, 20, 33, 40, 22, 28, 30, 24. Average, 30.
Rate per hour in oestrus:
Ovarian end: 30, 35, 28, 11, 11, 29, 18, 22, 37, 18, 20, 14, 23. Average, 26.
Vaginal end: 27, 9, 8, 8, 14, 11, 15,—,27, 13, 13, 15, 12. Average, 20.

Clark, Knaus and Parkes (6) showed that the ends contract independently. Thus the separation of the two ends eliminate the peristaltic influence of one end upon the other. As seen above the frequency of the rhythmic contractions is greater in dioestrus than oestrus. This finding agrees with that of Frank, Bonham, and Gustavson (9) in regard to the entire excised uterus. This is illustrated in the graphs, which are quite typical.

Ogata (8) found that all parts of the uterus show automatic rhythmicity no matter how small a part be taken. In this study it was found that the bisected uterine horn shows the greater frequency of rhythmic contractions at the ovarian end both in dioestrus and in oestrus. Of thirty animals only one showed greater frequency in the vaginal end. The relative rates of contraction are shown in the graphs.

The difference in the rate of contraction of the intact excised uterus in

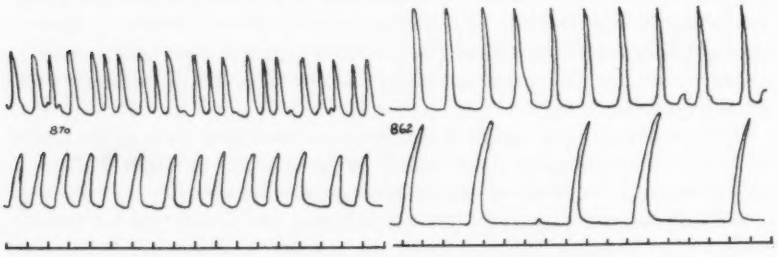

Fig. 1 Fig. 2

Fig. 1. Rhythmic contractions of isolated rat uterus in dioestrus. Above, ovarian end; below, cervical end. Time, 1 minute.

Fig. 2. Rhythmic contractions of isolated rat uterus in oestrus. Above, ovarian end; below, cervical end. Time, 1 minute.

oestrous and dioestrus has been shown by Frank, Bonham and Gustavson (9) to be due to the fluctuation in the ovarian hormone secretions, the greater amount secreted during oestrus lowering the rate of uterine contractions. Our work has confirmed these observations and shown further that segments of the bisected horn behave in the same way. As shown by one of us, (10) ovarian extracts, when placed in the bath containing isolated uteri, decrease the rate of contraction and occasionally the amplitude. This is further proof of the theory of Frank and his collaborators referred to above (9). It seems clear that in the normal animal the rate of contraction of uterine musculature is affected by the ovarian hormones.

The different rates of contractions of the two ends of the uterus may be considered in the light of gradient axiate structure. Childs (11) suggested

that the activity of the axiate structure, whether the entire organism or only an axiate structure in a more complex organism, is controlled by a gradient of metabolism. In a tubular organ with a muscular wall there exists a gradient muscular activity showing a metabolic gradient by its independently higher rate of contraction at the ovarian end while the lower rate is at the vaginal end. It is of interest that the hormone affects chiefly the rate of contraction but has no appreciable effect on the amplitude. Why this is so is problematical and is to be given further investigation.

SUMMARY

1. There seems to be a relation between the frequency of contractions and the stage of oestrus, in the bisected uterus.

2. There is a different rate of contraction of the two ends of the uterus in oestrus and in dioestrus.

3. Each end is independent as to the rate and type of contractions.

4. No correlation was found between the amplitude of contractions and the stages of oestrus.

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EMOTIONAL HYPERCHOLESTEROLEMIA

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An increasing recognition of the possible importance of the metabolism of the animal sterols has made it worth while to determine the rôle of the sympathetic nervous system in the regulation of the cholesterol level in the blood of the cat.

The sterols are not related to the fats in chemical structure, but many attempts have been made to correlate the metabolism of the two. Knudson (1917) has observed that after the ingestion of fat the ratio of cholesterol esters to free cholesterol in the blood rises, which may indicate that cholesterol acts as a conveyor of fatty acids.

Rakestraw (1921) found no alteration of cholesterol in the blood following exercise.

Conflicting statements as to the blood cholesterol level following splenectomy are reported. An increase has been found in dogs by some (King, 1914; Soper, 1915; and Leites, 1927), and not by others (Bodansky, 1925; and Leites, 1927). Splenectomy does not alter the level of the blood cholesterol in the rat (Randles and Knudson, 1928). Hypocholesterolemia following splenectomy in dogs has also been noted (Goebel and Gnoinsky, 1927). Soper (1915) feels that the changes following splenectomy are attributable to decreased elimination of cholesterol, possibly because of the loss of the splenic reticuloendothelial system.

The blood cholesterol tends to be diminished in hyperthyroidism (Denis, 1917; and Bing and Heckscher, 1924 and 1925), and elevated in hypothyroidism (Denis, 1917; and Heckscher, 1925). No change was found after suprarenalectomy in rabbits (Baumann and Holly, 1923) or rats (Randles and Knudson, 1928), but an increase has been observed in dogs (Joelson and Shorr, 1924). Castration does not influence the blood cholesterol in rats (Randles and Knudson, 1928).

Hypercholesterolemia has been found in cats following a large intake of cholesterol (Gardner and Lander, 1913).

Results following injections of epinephrine have not been consistent. Essinger and György (1924) report two cases on which cholesterol determinations were made forty-five minutes and twenty-four hours after

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subcutaneous injections of adrenalin in man. In one no change was found, but the other showed a slightly increased blood cholesterol at the end of the forty-five minute period. Löw and Pfeiler (1928) report a series of experiments on dogs in which five animals were given 0.2 mgm. of epinephrine hydrochloride per kilo. Blood cholesterol determinations before injection, two hours after, and four hours after, showed a transient elevation of the cholesterol level, most marked at the end of two hours in four of the five cases. Mazzeo (1928) reports a rise of blood cholesterol following injections of adrenalin in rabbits. Papilian and Jiano (1928) gave 2 cc. of pilocarpine to each of two dogs and noticed a rise of cholesterol in the blood that was concluded within one hour. Himwich (1931) has found irregular alterations of the level in response to adrenalin.

The present research was undertaken in an effort further to evaluate the foregoing data.

METHOD. The colorimetric method of cholesterol determination devised by Myers and Wardell (1918) was adopted. The standard was checked at regular intervals against weighed amounts of cholesterol subjected to the same routine procedure that was applied to whole blood. By repeated standardization it was possible to maintain an accuracy of 3 per cent. Whole blood for analysis was obtained by cutting the marginal vein of a shaved ear of a cat and collecting it in oxalated glass tubes. Quantities for analyses were measured in standardized Ostwald pipettes. All cholesterol determinations were done in duplicate.

A maximal stimulation of the sympathetic nervous system was obtained in two ways. Three experiments (I, II, and III) were performed on animals excited by fastening them to the cat-board and then shaking the board. In all other experiments the animal was exposed in a wire cage to a group of barking dogs. Hair erection, pupillary dilatation, increased pulse rate, and defense reactions were constantly observed in the normal animals. The sympathectomized animals, similarly exposed, exhibited defense reactions but did not show signs of sympathetic stimulation.

Throughout these experiments all animals were deprived of food for at least six hours prior to the beginning of the experiment. This rules out the possibility of an alimentary hypercholesterolemia.

RESULTS. *Normal controls.* In three normal cats blood cholesterol determinations were made during a two-week period at intervals of three to four days. About the middle of this period from four to six samples of blood were withdrawn, at ten-minute intervals, from each cat without exciting the animal. Cholesterol determinations in these samples remained within a 3-per cent limit of error. The maximum variation of the cholesterol level in the blood during the two-week period was 6.5 per cent.

Excitation of normal cats. Experiments I through VII were performed on normal male and female cats. The period of excitation averaged

5 minutes. In each of five of these seven experiments two readings were sufficiently elevated to exclude the error of the method as a cause. Experiments IV and V have only one such reading. These experiments reveal an average elevation of blood cholesterol of 27.6 per cent, reaching a peak in a period 20 to 40 minutes following excitation. Experiments VIII and IX show the usual rise following excitation, but this is followed by a transient depression of the cholesterol level. Both experiments were performed upon the same cat. No explanation is offered.

Excitation of sympathectomized cats. Experiments X through XIII were performed on sympathectomized animals. In three of the four experiments two preliminary determinations were made. The blood cholesterol level shows a questionable or slight immediate elevation without subsequent rise. No significance is attached to the slight preliminary elevation.

Excitation before and after cholecystectomy. In experiment XIV a normal cat was kept under full ether anesthesia for 15 minutes. Two days later the usual excitation caused the normal transient elevation of blood cholesterol. Two weeks later the gall bladder was removed, and after the usual post-operative care the experiment was repeated. Experiment XV shows that, after a longer period of excitation, the percentage rise of cholesterol occurs as usual but is slightly increased.

Experiment I. Normal male cat, February 5, 1929.

Time	*Mgm. per cent*	*Per cent change*
5 minutes before	102.2	
Period of excitation	5 minutes	
7 minutes after	114.3	12.8
30 minutes after	121.2	18.8

Experiment II. Normal male cat, February 22, 1929.

Time	*Mgm. per cent*	*Per cent change*
35 minutes before	106.6	
Period of excitation	5 minutes	
5 minutes after	121.2	13.7
30 minutes after	135.6	27.0
60 minutes after	105.4	

Experiment III. Normal male cat, March 2, 1929.

Time	*Mgm. per cent*	*Per cent change*
5 minutes before	107.2	
Period of excitation	5 minutes	
5 minutes after	117.3	9.3
15 minutes after	129.0	20.3
30 minutes after	148.7	38.5
45 minutes after	115.3	7.5
60 minutes after	110.1	

Experiment IV. Normal male kitten, May 29, 1929.

Time	*Mgm. per cent*	*Per cent change*
20 minutes before	110.5	
10 minutes before	109.9	
Period of excitation	5 minutes	
10 minutes after	114.9	4.2
40 minutes after	137.9	25.1
70 minutes after	114.3	3.7
100 minutes after	112.4	
130 minutes after	111.1	

Experiment V. Normal female cat. November 20. 1929.

Time	*Mgm. per cent*	*Per cent change*
15 minutes before	104.7	
Period of excitation	5 minutes	
25 minutes after	136.1	29.9
55 minutes after	105.8	

Experiment VI. Normal female cat, January 16, 1930.

Time	*Mgm. per cent*	*Per cent change*
5 minutes before	75.0	
Period of excitation	3 minutes	
25 minutes after	88.2	17.6
35 minutes after	87.7	16.9
90 minutes after	76.1	

Experiment VII. Normal male cat, January 25, 1930.

Time	*Mgm. per cent*	*Per cent change*
5 minutes before	95.2	
Period of excitation	5 minutes	
20 minutes after	129.9	36.4
35 minutes after	128.0	34.4
60 minutes after	96.1	
90 minutes after	97.1	

Experiment VIII. Normal male cat, March 12, 1929.

Time	*Mgm. per cent*	*Per cent change*
15 minutes before	112.0	
Period of excitation (escaped from cage)	5 minutes	
5 minutes after	133.3	19.0
15 minutes after	153.2	36.7
30 minutes after	174.6	55.8
45 minutes after	too low to read	
60 minutes after	too low to read	
75 minutes after	94.7	−15.5

Experiment IX. Same cat as experiment VIII, March 21, 1929.

Time	*Mgm. per cent*	*Per cent change*
5 minutes before	110.1	
Period of excitation	3 minutes	
5 minutes after	112.0	
15 minutes after	117.9	7.0
30 minutes after	142.8	29.7
45 minutes after	66.4	−40.0
60 minutes after	83.3	−24.4
75 minutes after	94.6	−15.1
105 minutes after	110.1	
135 minutes after	109.5	

Experiment X. Cat 403, sympathectomized, April 18, 1929.

Time	*Mgm. per cent*	*Per cent change*
25 minutes before	114.6	
10 minutes before	114.3	
Period of excitation	5 minutes	
5 minutes after	118.0	
15 minutes after	116.7	
30 minutes after	115.3	
50 minutes after	114.6	
70 minutes after	114.3	
95 minutes after	114.6	
125 minutes after	114.3	

Experiment XI. Cat 465, sympathectomized, May 6, 1930.

Time	*Mgm. per cent*	*Per cent change*
5 minutes before	95.2	
Period of excitation	5 minutes	
30 minutes after	95.2	
60 minutes after	95.2	

Experiment XII. Cat 408, sympathectomized, April 24, 1929.

Time	*Mgm. per cent*	*Per cent change*
20 minutes before	106.1	
10 minutes before	106.1	
Period of excitation	5 minutes	
5 minutes after	111.1	4.7
15 minutes after	108.1	
35 minutes after	107.3	
50 minutes after	106.7	
65 minutes after	106.4	
95 minutes after	106.4	

Experiment XIII. Cat 107, sympathectomized, November 30, 1929.

Time	*Mgm. per cent*	*Per cent change*
25 minutes before	97.1	
10 minutes before	97.5	
Period of excitation	5 minutes	
15 minutes after	97.5	
30 minutes after	98.0	
45 minutes after	98.0	
60 minutes after	97.5	

Experiment XIV. Normal cat. February 6, 1930: Etherized for 15 minutes. February 8, 1930: Experiment below.

Time	*Mgm. per cent*	*Per cent change*
10 minutes before	95.2	
Period of excitation	5 minutes	
30 minutes after	117.6	23.5
45 minutes after	105.3	10.6
60 minutes after	97.5	
80 minutes after	94.8	

Experiment XV. Same cat as experiment XIV. February 20, 1930: Cholecystectomy under ether. February 28, 1930: Experiment below.

Time	*Mgm. per cent*	*Per cent change*
20 minutes before	95.2	
5 minutes before	94.3	
Period of excitation	10 minutes	
5 minutes after	103.1	8.7
20 minutes after	128.0	35.0
35 minutes after	121.2	27.7
50 minutes after	113.6	19.8
65 minutes after	105.3	11.0
95 minutes after	95.7	
110 minutes after	96.1	

COMMENT. It seems evident from these experiments that sympathetic stimulation in the cat is associated with a transient hypercholesterolemia. This is represented in a peak rise at the end of about half an hour, with a maintained return to normal about an hour after the excitation. This emotional hypercholesterolemia does not occur in sympathectomized animals.

Notwithstanding abundant evidence against contraction of the gall bladder by sympathetic stimulation, it was thought desirable to disprove experimentally the possibility that the hypercholesterolemia was attributable to the resorption of cholesterol after the discharge of a cholesterol-rich bile into the upper intestine.

It is of interest that the elevation of blood cholesterol is strikingly similar to the elevation of blood sugar in emotional hyperglycemia. Similar elevation of sugar and cholesterol in the blood is also known to occur in diabetes mellitus.

As to the significance of emotional hypercholesterolemia, little can be said. There is no good evidence to show that cholesterol plays any part in immune reactions, although that idea has been suggested by various authors. There is, furthermore, little or no evidence that arteriosclerosis is attributable to faulty cholesterol metabolism. No useful or deleterious function can be assigned at present to this transient elevation of blood cholesterol.

CONCLUSIONS

Normal cats adapted to a routine laboratory life show a fairly constant blood cholesterol level.

The experimental excitation of a normal cat produces an emotional hypercholesterolemia. Within 20 to 40 minutes after excitation there is an increase in the blood cholesterol of 25 to 30 per cent. The percentage usually returns to normal within an hour and is maintained subsequently at that level.

Sympathectomized animals show no emotional hypercholesterolemia.

Cholecystectomy does not abolish emotional hypercholesterolemia.

It is a very real pleasure to express my indebtedness to Dr. W. B. Cannon for his helpfulness in the planning and direction of these experiments.

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STUDIES IN CARDIAC PERMEABILITY[1]

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When hearts of rabbits and dogs are perfused, even with hypertonic solutions, their weight can be made to become more than double if the hydrogen-ion concentration of the perfusing solutions is suitable. This will also occur even though these solutions may contain considerable quantities of hydrophilic material such as gum acacia and starch with huge molecular aggregates or colloid micellae as their units of dispersion. From physiochemical considerations it was concluded (Ort and Markowitz, 1931) that these materials actually penetrated into the perfused hearts. However, Walker and Keith (1930) have shown that when gum acacia is injected intravenously into dogs it is present for days in the circulation. Indeed it is assumed that the gum acacia can pass but very slowly through any of the normal body tissues. The success of the replacement of costly blood transfusions to combat surgical shock, by intravenous injections of 6 per cent gum acacia solutions in saline depends on the facts that the kidney cannot rapidly excrete the gum acacia and that the gum acacia cannot pass readily into the body tissues generally. Since the trapped gum acacia exerts some kind of an attraction for the water in which it is suspended, this too is kept in the circulation, with the result that the blood volume is raised and maintained at a level which insures the desired blood pressure. These sequelae are in distinct contrast to those following injections of simple saline or Ringer-Locke's solutions, for the elimination of water by the kidneys soon prevents these solutions from giving more than temporary relief.

It was the purpose of the present investigation to study the causes of the increased permeability of the perfused hearts and to see if these same factors would be effective also in the intact animal organism. Gum acacia was again used, this time as a test object to detect changes in permeability in the hearts. The gum acacia micellae are very convenient for this purpose as their size is such as to prevent them from rapidly penetrating into the interior of normal hearts while they can readily enter into perfused hearts and into hearts in intact animals under conditions to be described.

[1] Read before the Biochemical Division of the American Chemical Society, Cincinnati, Ohio, September, 1930.

Moreover, the presence of the gum acacia in hearts can be easily determined in a roughly quantitative manner. This was done by the use of Bial's orcinol-hydrochloric acid reagent for pentoses (Hawk, 1923). Saline extracts of the heart tissues after precipitation with sulphuric acid and sodium tungstate, and the syneresis fluids coming out of the tissues on standing were used for the determinations.

All heart perfusions were made in the usual manner which has been previously described (Ort and Markowitz, 1930), withdrawals of blood and injections of the various solutions were done at an external jugular vein or at a carotid artery. Immediately after the end of an experiment the entire heart if small, cut open in several directions, or a portion removed from its apex if large, is firmly squeezed and rolled in a dry towel for a few moments to remove extraneous fluid, including that in the blood vessels, except perhaps some in the capillaries. This latter was considered to be balanced by controls treated in exactly the same manner. The heart tissue thus obtained was then placed in a tightly corked specimen bottle and allowed to stand on ice at least until the next day. This was done to see if syneresis of the tissue colloids would take place. It was found that whenever any considerable amount of free liquid accumulated in the bottle with the hearts as a result of syneresis, gum acacia was usually found both in the liquid and in the shrunken heart tissue remaining.

Tissue from a normal dog's heart, tissue from a rabbit's heart following one and a half hours of deep ether anesthesia, and tissue from a rabbit's heart which had been perfused for seventy-eight minutes with ordinary Ringer-Locke's solution containing no gum acacia have present in them a small amount of material which gives a slight blank reaction for a pentose. In terms of gum acacia this amounts to about 1 mgm. of gum acacia for each gram of heart tissue (expts. 1, 2 and 3: each experiment in the tabulation must be considered as a typical or average experiment selected from among others the results of which were essentially the same).

We repeated a former experiment (Ort and Markowitz, 1931) in which a rabbit's heart was perfused for two hours with Ringer-Locke's solution to which had been added 9 per cent gum acacia (expt. 4). Although this solution was hypertonic the heart more than doubled its weight. When the heart was allowed to stand, after the experiment, more fluid rich in gum acacia (27 mgm. for each cubic centimeter) came out of the heart than the original weight or volume of the heart before the experiment. This was in spite of the fact that immediately before placing the heart in the specimen jar it was squeezed and wiped quite dry. Even after the capillaries were flushed out by the extrusion of this comparatively great volume of liquid, the shrunken tissue remaining gave a strong reaction for the gum acacia (6.5 grams for each gram of tissue; the figure 47.2 is based on the weight of tissue remaining and is the sum of the gum acacia in the tissue and that in

TABULATION

Illustrative experiments

EXPERIMENT	ANIMAL	PROCEDURE	TOTAL GUM ACACIA, MGM. FOR EACH GRAM OF TISSUE
1	Normal dog		0.9
2	Rabbit	Deep ether anesthesia	0.8
3	Rabbit	Heart perfused with Ringer-Locke's solution	1.3
4	Rabbit	Heart perfused with 9 per cent gum acacia in Ringer-Locke's solution	47.2
5	Rabbit	Heart perfused with 6 per cent gum acacia in Ringer-Locke's solution	25.0
6	Dog	Heart perfused with 6 per cent gum acacia in Ringer-Locke's solution	33.0
7	Dog (12 kgm.)	400 cc. 6 per cent gum acacia in Ringer-Locke's solution injected intravenously	1.5
8	Dog (15 kgm.)	410 cc. blood withdrawn and replaced by 500 cc. 6 per cent gum acacia in Ringer-Locke's solution	2.5
9	Dog (6 kgm.)	Deep ether anesthesia; 150 cc. blood withdrawn and replaced by 250 cc. 6 per cent gum acacia in saline	2.5
10	Dog (10 kgm.)	Etherized; thorax opened; 170 cc. blood withdrawn; 500 cc. 6 per cent gum acacia in Ringer-Locke's solution added at start and 150 cc. 12 per cent fifty-three minutes later	3.4
11	Dog (10 kgm.)	Etherized; thorax opened; heart denervated; 900 cc. 6 per cent gum acacia in Ringer-Locke's solution added	2.7
12	Dog	Etherized; blood repeatedly withdrawn and replaced by 6 per cent gum acacia in Ringer-Locke's solution until erythrocytes constituted 1.3 cc. for each 15 cc. of blood (8.7 per cent)	11.0
13	Dog	Heart-lung preparation; 6 per cent gum acacia in Ringer-Locke's solution and heparinized dog's blood mixed so that erythrocytes constituted 1.3 cc. for each 15 cc. of blood (8.7 per cent)	12.8
14	Dog	Heart-lung preparation similar to that in experiment 13 except erythrocytes constituted 3.5 cc. for each 15 cc. of blood (23 per cent)	3.5
15	Dog	Similar to experiment 12, except blood withdrawn was replaced by 6 per cent gum acacia in plasma; erythrocytes constituted 1.4 cc. for each 15 cc. of blood (9.3 per cent)	10.3
16	Dog	Similar to experiments 12 and 15 except that replacement was with an erythrocyte suspension in 6 per cent gum acacia in Ringer-Locke's solution; erythrocytes constituted 5.0 cc. for each 15 cc. of blood (33 per cent)	3.7
17	Dog	Heart perfused with an erythrocyte suspension in 6 per cent gum acacia in Ringer-Locke's solution; erythrocytes constituted 6.3 cc. for each 15 cc. of blood (42 per cent)	5.0
18	Dog	Heart perfused with a solution of 6 per cent gum acacia in plasma	22.0

the syneresis fluid). These facts we regard as proof of the penetration of gum acacia into the perfused rabbit's heart. The behavior of small dogs' hearts perfused in a like manner is similar.

Since 6 per cent gum acacia in saline is the strength commonly used intravenously to combat shock, this concentration was chosen for the remainder of the work. Accordingly, hearts from rabbits and small dogs were next perfused for one and a half hours with a 6 per cent solution of gum acacia in Ringer-Locke's solution (expts. 5 and 6). These hearts also gained weight during the perfusion, and syneresis occurred when the tissue stood overnight. Both fluid and shrunken tissue remaining gave strong reactions for gum acacia. As might be expected, the total gum acacia in the hearts, based on the weight of the shrunken tissue remaining, was less than that found when a 9 per cent gum acacia solution was used and the perfusion time was two hours (expt. 4).

A series of control experiments was next run to show that the presence of considerable gum acacia in the circulation of more or less normal animals does not necessarily mean that any appreciable quantity of it will enter the heart. A simple intravenous injection, seventy minutes before the animal was killed (expt. 7), an intravenous injection following the withdrawal of a considerable quantity of blood (animal was allowed to live one and a half hours thereafter, expt. 8) and a similar experiment in which the dog was kept for the one and a half hour period after the injection of the gum acacia under ether anesthesia as deep as possible with a living animal (expt. 9), all failed to cause appreciable penetration of the gum acacia into the heart muscle. This was also the case even when the thorax was opened under ether anesthesia and its contents manipulated considerably by the hands (expt. 10). To the operations of this last experiment was next added the complete denervation of the heart. Both vagi were cut in the neck or in the thorax near the heart. The sympathetic innervation to the heart was also destroyed by bilateral removal of the stellate and the adjacent thoracic ganglia (expt. 11). The experiment was terminated one hour after the complete denervation of the heart. The heart from this dog had about as much gum acacia in it as was found in those from the control dogs (expt. 10) in which the animals had been likewise anesthetized with ether and the thorax was opened.

In all these control experiments there was a similar irreducible minimum of liquid remaining in the capillaries after squeezing and rolling the tissues in dry towels. Therefore it is apparent that the far greater amount of gum acacia found in the perfused hearts (expts. 4, 5 and 6), and in some of the experiments to be described, is in them because they are more permeable to gum acacia than the controls described. The increased permeability of the isolated perfused hearts is not, however, due to denervation.

Experiments were next performed in which the removal of blood from lightly anesthetized dogs followed by the injection of 6 per cent gum acacia in Ringer-Locke's solution was repeated until the erythrocytes remaining were only a small fraction of their original amount. When these dogs were killed by bleeding from the carotid artery after one and a half hours, the hearts were obviously swollen. Portions were removed from the apex and squeezed in a towel and wiped dry as usual. Nevertheless liquid was shortly exuded which was rich in gum acacia, and saline extractions of the remaining heart tissue also were strongly reactive (expt. 12).

Experience showed that when the dilution of the blood had been carried to the extent that there were less than 2 cc. of erythrocytes in 15 cc. of centrifuged blood (13 per cent) considerable gum acacia would be found in the hearts. If much more than 2 cc. were left, only a little would enter. These same observations held true when the circulation was limited to a heart-lung preparation (expts. 13 and 14). The denervation and removal of the other organs and tissues from any connection with the circulation did not change the picture.

We next separated the blood into its two components, erythrocytes and plasma, by bleeding into heparin to prevent clotting and then centrifuging. The cells were next washed in Ringer-Locke's solution and centrifuged again. Suspensions of the washed cells were made in Ringer-Locke's solution containing 6 per cent gum acacia, the cells constituting about 3 to 5 cc. for each 15 cc. (from 20 to 33 per cent). The plasma and gum acacia were added to Ringer-Locke's solution to make a mixture of 50 per cent plasma and 6 per cent gum acacia. Experiments of repeated removals of blood followed by injections of one or the other of these mixtures were then made on other dogs similar to the way in which the simple 6 per cent gum acacia had been used. When the plasma solution was used (expt. 15) nearly as much gum acacia was found in the heart as when 6 per cent gum acacia in Ringer-Locke's solution alone was used (expt. 12), although the content of plasma in the circulating fluid was not greatly changed. The volume of erythrocytes was also a little higher, 1.4 cc. for each 15 cc. (9.3 per cent) than in experiment 12. However, when the cell suspension was used (expt. 16) not much more gum acacia was found in the hearts than after a simple intravenous injection of gum acacia into anesthetized dogs. Five consecutive removals of blood, followed by injections of the cell suspension, reduced the plasma content to nearly a tenth of its normal value, and left the circulating fluid about 5 per cent gum acacia. If this procedure is carried too far, the heart becomes edematous, but the fluid exuding from a piece of it on standing contains only a small amount of gum acacia.

Apparently, then, even in the intact animal the absence of sufficient erythrocytes causes heart tissue to become more permeable. Hence also in perfusions with Ringer-Locke's solution the hearts are more permeable than

normal hearts because of the absence of erythrocytes from the perfusing fluid. To test out this conclusion hearts of small dogs were perfused with the erythrocyte suspensions in a medium of gum acacia and Ringer-Locke's solution. These hearts had but little gum acacia in them (expt. 17) compared to those perfused with 6 per cent gum acacia in Ringer-Locke's solution alone (expt. 6). When other dogs' hearts were perfused with the plasma solutions, considerable penetration of gum acacia and some edema resulted, even though the perfusing solution was hypertonic (expt. 18).

Since it is thus established that cardiac permeability is influenced by the presence or absence of erythrocytes, the questions naturally arise: Is the oxygen-carrying capacity of the erythrocytes the property which is involved? Does oxygen-lack tend to increase the permeability of tissues in general? Although we hope to present evidence on this phase of the problem in a subsequent paper we can state here that as far as cardiac permeability is concerned we believe the oxygen tension in the tissues to be a significant factor.

The lack of erythrocytes, then, is apparently the factor in increasing the permeability of perfused hearts over that of normal hearts. The same effect can be accomplished in the intact animal by providing a circulating fluid with few erythrocytes. Concentration of plasma seems not to be so very important in cardiac permeability. Complete denervation of the heart is also without much effect in acute experiments. It is suggested that the oxygen-carrying capacity of the erythrocytes is the factor involved.

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THE RELATIONSHIP BETWEEN FOOD AND WATER INTAKES IN MICE[1]

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There are several reports in the biological literature to the effect that some mice can thrive without drinking, even when they are supplied with only air-dried foods containing not more than 10 per cent of moisture. Some of these statements, which are in striking opposition to the old maxim, *corpora non agunt nisi fluida*, have not been presented with any experimental evidence in their support (Babcock, 1912; Forbes and Keith, 1914). The data of Gates (1926) are not very extensive, although he reported that some mice not only lived on dry foods but were also able to reproduce and to rear one or two young per litter. One wonders whether the young mice that were not reared may not have served as a hidden water supply. Bailey's experiments (1923) were done with two species of pocket mice (*Perognathus sp.*) that ordinarily inhabit certain regions of North Dakota. His account does not indicate whether the lack of water or the restriction to a diet of mixed seeds was responsible for the decline of the animals after six weeks. Although the possibility that animals will live several weeks without water is impressive, the ability of mice to thrive under conditions of water deprivation still remains to be proved.

The mouse, like many other animals, does not possess generally distributed sweat glands; it does not rely upon evaporation through the skin to regulate body temperature, and therefore should present a simpler case for the study of the elusive problem of water requirements than does man. The experiments reported herein were planned to study the water intake of mice under various conditions, and to test the assumption that these animals can live without drinking. In brief, our results have shown that the deprivation of water is followed by an immediate drop in the quantity of food ingested, and death is due to dehydration and starvation. Under various conditions the intake of dried food appears to be about the same

[1] Many of the data in this paper are taken from a dissertation presented by Franklin C. Bing in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Yale University, 1930.

in weight as the amount of water drunk, or somewhat less. In addition to the data relating food and water intakes some observations were made of the body temperatures of normal and dehydrated mice.

EXPERIMENTS. *The voluntary food and water intakes of adult mice.* Three groups of vigorous adult mice that had been reared on stock rations were used. Except the second group, which consisted of ordinary "white" mice, the animals were of the inbred strain of Bagg albino mouse originally obtained from the Cold Spring Harbor Station for Experimental Evolution. The animals were maintained in individual cages, supplied with drinking pipets, and described elsewhere (Bing, 1931). The compositions of the diets fed are presented in table 1. Diet A was a stock ration that has been found suitable for growth and reproduction through several generations. Diet B was simply a mixture of canary, rape and hemp seeds. Obviously, a diet of seeds would not be satisfactory for long periods due to the mineral and vitamin deficiencies, but over a relatively short time it might be considered usable for adult animals. The object of using this ration was to ascertain whether a more or less "natural" diet, with foods of low moisture content in their native state of dehydration, might result in successful maintenance without water. In addition, the physical texture of the seeds appeared to be better suited for ingestion than the powdered mixture represented by diet A. Diet C was designed to contain minimum amounts of protein and inorganic salts, without being deficient in either respect. Preliminary experiments showed that 5 per cent of casein in a ration that also contained 25 per cent of fat resulted in the maintenance of body weight of both young and adult mice for considerable periods of time, and also that 0.5 per cent of McCollum's 185 salt mixture permitted fair growth and produced histologically normal bones in young mice.[2] The diet C, therefore, may be regarded as a ration furnishing the least amount of protein and a complete salt mixture consistent with good nutrition at least for a much longer time than the period of these experiments. While the inclusion of yeast and cod liver oil is not a desirable practice, we felt that in the present instance it would not be objectionable, provided that the diet were made up frequently and used with adult mice for only short periods of four to ten days.

On each of the three diets the mice maintained body weight during the periods of observation. The average food and water intakes of each group are presented in table 2. They show that regardless of the nature of the diet the amount of water drunk was proportional to the weight of dried food ingested. The lower intake of diet B can be related to the higher caloric value of the fatty seeds. On this ration the water intake was also low, but the ratio of water to food remained unaltered. Although diet C furnished appreciably less urea and salts, for the excretion of which the

[2] Unpublished experiments.

body is deprived of water, the fluid consumption was not much reduced below the level of the intake on the stock diet.

Effects of deprivation, and subsequent restricted intakes of water. The water tubes were removed from the cages and the records of body weight and food consumption continued as before. The results of body weight

TABLE 1

Composition of the diets

DIET NUMBER	CONSTITUENT	GRAMS PER 100	ANALYSIS	GRAMS PER 100
A	Dried whole milk	50.0	Moisture	9.6
	Ground whole wheat	47.4	Ash	4.5
	Sodium chloride	1.4	Fat (ether extract)	14.0
	Calcium carbonate	1.2	Protein (N x 6.25)	18.5
			Carbohydrate	53.4
B	Whole hemp seed	(The seeds were simply mixed and fed and the mice ate their choice)		
	Rape seed			
	Canary seed			
C	Casein	5.0	Moisture	8.0
	Lard	20.0	Salts	0.5
	Cod liver oil	5.0	Fat	24.5
	Salt mixture*	0.5	Protein	4.5
	Dried yeast	5.0	Carbohydrate	62.5
	Sucrose	64.5		

* McCollum's salt mixture 185 was used. Journ. Biol. Chem., 1918, xxxiii, 55.

TABLE 2

The voluntary food and water intakes of adult mice

GROUP	DIET	NUMBER OF MICE	AVERAGE WEIGHT	AVERAGE FOOD INTAKE	AVERAGE WATER INTAKE	RATIO OF WATER TO FOOD
			grams	*grams*	*cc.*	
1	A	10	23.6	3.2	4.1	1.3
2	A	8	22.4	3.5	4.4	1.2
2	B	8	22.4	2.1	2.7	1.3
3	C	11	23.1	3.0	3.2	1.1

measurements of mice fed diet A are shown graphically in figure 1. They indicate that each mouse lost weight promptly and rapidly when deprived of water. Those that lost more than about 33 per cent of their weight died, usually within 4 to 7 days. Although accurate records of the food ingested were difficult to secure it seemed that the animals decreased their food intake on the day the water was removed, and developed complete ano-

rexia in two days. Death was due, therefore, to lack of food as well as lack of water. There was no evidence that would lead one to suppose that these mice could live upon the stock diet without access to water.

The mice fed diet A were kept without water for 4 days, except mouse 308 which was deprived for 5 days, and then measured volumes of water

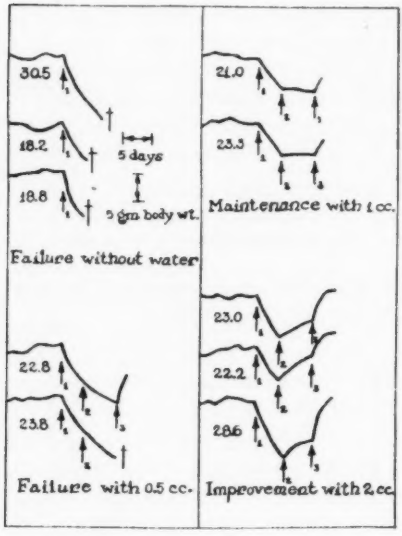

Fig. 1

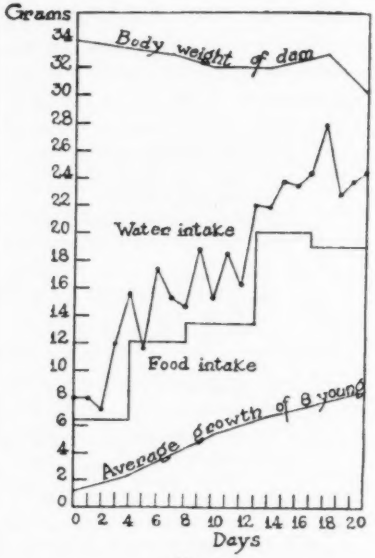

Fig. 2

Fig. 1. The changes in body weight of mice fed upon diet A are shown. In the first ten days free access to food and water was permitted. At the times indicated by the first arrow, water was removed; by the second arrow, measured volumes of water were administered; by the third arrow, water was again made freely available. The weight in grams of each mouse at the beginning of the experiment is also indicated. The results show that under the conditions described in the text, 1 cc. of water enables the mouse to maintain body weight at a sub-normal level.

Fig. 2. The food and water intakes of a lactating mouse are shown. The water drunk was measured daily; the food intake was weighed at convenient intervals. The eyes of young mice open on the thirteenth day; the young begin to eat solid food on the fifteenth day of life. The chart illustrates that the water intake is proportional to the amount of food eaten at all times during the lactation period.

were given to each animal in one dosage daily. The amounts administered were 0.5, 1.0 and 2.0 cc. Three animals were continued without water to serve as controls. The results showed that mice continue to lose weight when given 0.0 and 0.5 cc., are able to maintain weight when given 1.0 cc., and to gain a little when given 2.0 cc., of water. Our figures for

food consumption indicated that with an increased water allowance more food was ingested. The results may be interpreted as indicating that under the conditions of decreased metabolism following fasting, 1.0 cc. of water permits the mouse to eat sufficient calories in the form of the stock diet to maintain body weight at a sub-normal level.

The mice fed diet B also lost weight when deprived of water. On the sixth day two mice died and the remaining animals were in wretched condition. When given graded amounts of water—0.5, 1.0, 1.5 and 2.0 cc. daily—maintenance of body weight was obtained with 0.5 cc., and some increase in weight noted with the larger volumes. The smaller amount of water required for maintenance illustrates the influence of the nature of the diet upon the water requirements. In this case, the drinking of 0.5 cc. of water permitted a small amount of dried food to be eaten, and the caloric value of the ingested food was sufficiently high to result in maintenance of weight under the conditions imposed.

Similar results were obtained with the mice fed upon diet C. After three days of water deprivation graded amounts were given. Those not given water died and those given 0.5 cc. continued to lose weight and finally died. With 1.0 cc., maintenance for a period of 5 days (after which the experiment was discontinued), and with 2.0 cc., some slight growth resulted. This group of mice, therefore, almost precisely duplicated the results obtained with the mice fed upon the stock diet.

Observations of their behavior showed that all the animals deprived of water for several days would be dull and inactive in their cages until the water allowance was given. This was consumed at once and immediately afterwards each mouse would begin to eat a little food. The impression received was that the mice were unable to eat the dried food unless they had simultaneous access to water. It is interesting to note that in a study of the feeding and drinking habits of rats in specially constructed cages, Richter (1927) observed that his animals ate at regular intervals 6 to 10 times each day. Other records showed that rats drank at uniform periods about 10 times daily. Apparently, no experiments were made to relate the eating and drinking cycles to each other, but it seems reasonable to suppose that the animals ate and drank at about the same time. The diet used by Richter was a stock ration similar in texture and in moisture content to our own.

Room temperature and humidity. Because of the known effect of atmospheric conditions upon the water balance we deemed it advisable to record daily the minimum and maximum temperatures of the animal room, and each morning the dry and wet bulb readings, and the barometric pressure. During all the experiments recorded herein the lowest amount of moisture, expressed as milligrams of water vapor per liter of air, was 9.4, the highest, 21.5, and the average, 15.9. The lowest relative humidity recorded was 40

per cent, and the highest, 75 per cent. The lowest temperature recorded was 72°F., and the highest, 87°F. Thus, we may conclude that fluctuations in temperature and humidity were not unduly large.

Body temperature. The rectal temperature of each mouse in the group fed the stock diet A was recorded daily with a tested clinical thermometer, selected so as to be of suitable size. The bulb was 15 mm. long with a diameter of 2 mm. It was possible to insert the entire bulb into the rectum, hold it in place one minute, and note the maximum temperature. With practice, we were able to obtain such records while the animal remained perfectly quiet. The results showed that under normal conditions the temperature varied rather considerably from day to day. The average of 110 observations on 10 male mice was 37.8°C., the lowest temperature observed being 36.0°C. and the highest, 40.0°C. Sumner (1915) also found large variations in the rectal temperatures of mice, his observations ranging from 34.8°C. to 39.0°C.

When deprived of water, the body temperature of all the mice fell below normal. To quote only one example, the average temperature of mouse 308 during the period of free access to food and water was 38.6°C., with extremes of 37.3° and 40.0°. On successive days after deprivation of water the readings were 38.9, 39.1, 38.4, 37.2 and finally, 30.6°C. Immediately following the last observation 2.0 cc. of water were administered and the temperature noted on the next day was 36.2°C. These observations on the drop in body temperature are in agreement with those of Simonowitch (1896) on rabbits and guinea pigs, of Avrorov (1900) on dogs, and of Pucher (1928) on puppies, all deprived of food and water. They show that mice as well as other animals do not develop the so-called inanition fever that may be observed in babies.

Experiments with rats. In order to obtain further evidence of the relationship between food and water intakes some observations were made upon 6 adult rats, from the strain of the Connecticut Agricultural Experiment Station. Their intakes of stock diet A were recorded over a period of 10 days during which they were permitted free access to both food and water. No measurements of water intakes were made during this period because we were primarily interested in the condition following the first few days of inanition brought about by water starvation. The experimental procedure was similar to that followed with the mice, and the data are summarized in table 3. They indicate clearly that a quantitative relationship actually exists between the amount of water ingested and the food intake. One might say from these results that in order to eat 3 grams of the stock diet required to maintain body weight at a sub-normal level, the rat must have about 4 cc. of water. In order to eat twice as much dried food the water allowance must first be doubled.

The food and water intakes of lactating mice. Further evidence that tends

to link together water and food intakes was furnished by recording the amounts ingested by lactating mice. As a converter of energy the mouse has few equals. Some of our best records show that 30-gram-mothers may give birth to a litter of 10 young weighing 15 grams, and in 3 weeks rear all of their babies to a total weight of 120 grams, which is 4 times the weight of the mother. Such animals consume truly enormous quantities of food and water, so much so that the ordinary water tubes used in these

TABLE 3

The relation between food consumption and water drinking in rats

PERIOD	RAT NUMBER	FOOD PER DAY	WATER PER DAY	CHANGE IN BODY WEIGHT PER DAY
		grams	*cc.*	*grams*
Water *ad libitum* for 10 days	1	11.6	Not measured; can be estimated at 10–14 cc. per rat per day	+1.4
	2	9.8		+2.2
	3	8.7		+1.0
	4	9.9		+1.2
	5	8.4		+0.8
	6	9.7		+1.6
Water deprivation for 6 days	1	3.5	None	−8.3
	2	3.4		−7.8
	3	2.0	(All food was eaten in first 3 days only)	−6.8
	4	2.8		−8.1
	5	1.8		−6.5
	6	3.6		−8.5
Restricted water for 6 days	1	5.8	8.0	+1.5
	2	8.5	8.0	+2.0
	3	2.7	4.0	−0.0
	4	3.7	4.0	−0.8
	5	0.0	2.0	−1.7
	6	0.0	2.0	−4.2
Unrestricted water for 2 days	5	6.0	Not measured	+15.0
	6	14.0		+19.5

experiments were wholly inadequate for holding the necessary volume. For our present purpose, we have chosen a single representative record, indicating an average performance that can be readily duplicated. The data, presented graphically in figure 2, emphasize the close relationship that exists between water and food intakes. The mouse at the height of the lactation period ate enough stock diet to equal nearly two-thirds of its body weight, and drank somewhat more water. At any time during

the lactation period the water intake amounted to about 1.3 cc. per gram of ingested food.

Discussion. From the preceding data of the voluntary food intakes of diets of known composition it is possible to compute the metabolic end-products, the excretion of which brings about a loss of water. In table 4 are shown the values for the CO_2, H_2O and urea resulting from the utilization of the average ingested amounts of stock diet A and synthetic diet C. The principles upon which such calculations are based are given by Babcock (1912). The figures for the intakes are multiplied by the arbitrary factor of 0.9 to allow for digestibility. The excretion of the undigested residues and other products in the feces involves a certain loss of water. The water output is also dependent upon the amount of CO_2 to be removed by the lungs, and to the urea and salt content that must be eliminated

TABLE 4

Comparison of calculated water requirements with actual fluid intakes of mice

GROUP	DIET	METABOLIC END-PRODUCTS		CALCULATED WATER LOSS			
		CO_2	Urea	Lungs	Feces	Urine	Total
		grams	*grams*	*grams*	*grams*	*grams*	*grams*
1	A	4.46	0.18	1.12	0.70	3.00	4.82
3	C	4.59	0.04	1.14	0.70	0.67	2.51

GROUP	DIET	WATER SOURCES FROM FOOD		ACTUAL WATER INTAKE	FLUID INTAKE IN EXCESS OF CALCULATED REQUIREMENTS
		Metabolic water	Moisture		
		grams	*grams*	*cc.*	*cc.*
1	A	1.48	0.31	4.15	1.12
3	C	1.75	0.24	3.20	2.68

through the kidneys. It would appear that available data concerning the cutaneous water loss by animals lacking in sweat glands and covered with fur, such as the mouse, are very indefinite and for present calculations this excretion may be assumed to be small and constant in the different mice.

The pulmonary water loss may be estimated with some degree of exactness. This excretion cannot be controlled by the animal, and it is dependent only upon the volume of breathing, the temperature and humidity of the atmosphere and the temperature of the exhaled air. It may be assumed that the mouse inhales air at about 20°C., containing say, not more than 20 to 25 mgm. of water vapor per liter. The exhaled air is at a temperature somewhat below the body temperature of the mouse, and may be considered to be saturated with moisture at 35°C., or in other words to contain about 40 mgm. of water vapor per liter. The mouse under such conditions of humidity will lose about 20 mgm. of water for

each liter of air breathed. The volume of breathing is related to the metabolism (Y. Henderson, 1925) or the CO_2 production. If the exhaled air contains about 4 per cent of CO_2 then the volume of air breathed by the mouse on the stock diet will be 56 liters, and on the synthetic diet, 57 liters. This implies a water excretion by way of the lungs, in the first case of 1.12 grams, in the second, of 1.14 grams.

Theoretically, an atmosphere saturated with water vapor at 35°C. would not deprive the body of water during respiration. However, Hill and Macleod (1903) have shown that mice do not thrive under conditions of high atmospheric humidity.

The water loss by way of the intestines may be estimated at about 0.7 gram for each mouse. This figure is derived from the consideration that the dried weight of the feces is about 10 per cent of the intake of dried food (Dawburn, 1928) and that the feces contain in the neighborhood of 70 per cent water.

Many factors enter into the volume of the urinary water loss, but chief among these are the amounts of urea and salts that are excreted by the kidneys. If we assume that the concentration in which urea is ordinarily excreted is about 0.9 M, the water loss in the urine may be approximated. Gamble, Putnam and McKhann (1929) have shown that a rat consuming 67 mM of urea per day will drink 65 cc. of water, giving a urea concentration in terms of water intake of about 1 M. Actually, the concentration of urea in the urine under the conditions imposed by these investigators must have been considerably higher, so that our estimate of 0.9 M urea concentration in the urine may be considered low rather than high.

From the summary of these calculations presented in table 4 it is at once apparent that the water intake cannot be related to the estimated water loss. The water drunk by the mice on the two diets is in excess of the calculated requirements, and the corresponding urinary secretions must differ considerably in their concentrations of urea. Under ordinary conditions, therefore, the mouse does not regulate its water intake so as to excrete urea at a uniform concentration. It seems instead that the mouse drinks enough water to equal the weight of dried food consumed, provided this volume equals or exceeds the amount of water required to excrete the metabolic waste products. From a consideration of the observations recorded herein it may be suggested as a tentative explanation that the mouse drinks in order to moisten the dried food and render it capable of ingestion. The volume of water that appears to fulfill the lubrication requirements is about equal, or slightly more than the weight of dried food. In accordance with this interpretation one can understand why the mice on the synthetic diet, although from the standpoint of excretory requirements they do not need as much as the mice on the stock diet, yet drink

almost as much. In each instance the fluid intake roughly equals the weight of dried food.

Many data are available to support the conclusion that mice and rats consume water in amounts about equal to the weight of dried food consumed. The experiments of Rose, Stucky and Mendel (1930) show that the water consumption parallels the food intake in rats deprived of vitamin B and that the loss of appetite commonly observed in vitamin B deficient animals is accompanied by a decreased water intake. Moreover, these investigators showed that in both the depletion and recovery periods the volume of water drunk approximately equalled the weight of dried food eaten at all times. The data of Gamble, Putnam and McKhann (1928) show that in their basal periods the rats ingested 13.0 grams of dried food and 16.3 cc. of water. The ratio of water to food was 1.26, or about the same as we have found for mice. Such intakes of water certainly cover the requirements of ordinary normal diets, and it is a matter of common knowledge that animals can thrive upon foods that contain 50 to 60 per cent of moisture, without drinking.

Richter and Brailey (1928) have measured the water drunk by rats kept on a dried diet of constant composition over long periods of time. Their results showed that the fluid intake was greater with increasing body weight, and a very good correlation was found between the water intake and the body surface of the animals. Osborne and Mendel (1918) pointed out that the food intake of the rat could be related to the caloric value of the diet. Their observations have been abundantly confirmed for both rats and mice, and the food intake can therefore be related to the surface area of the animal, in accordance with Rubner's views. Thus, there is no essential difference between the conclusions of Richter and Brailey and our own. If the food intake is proportional to the surface area and the water intake is proportional to the amount of food ingested, then it follows that the water intake is also proportional to the body surface. But because of the lack of relationship between water drinking and total metabolism it would seem preferable to relate the water consumption simply to the food intake. If the rat and mouse eat for calories they drink in order to ingest dried food.

There is no evidence from our experiments that the mouse can thrive in the absence of water. From the calculations of the requirements for excretion and the total available water from the ingestion of dried food, it would seem as though only the barest possibility exists for the mouse to live in the absence of drinking water. It may be that species of mice that ordinarily inhabit arid regions might be able to ingest dry foods in sufficient quantity to maintain the requirements for nutriments, calories and water, but our data would suggest that in the absence of water anorexia would probably develop. Certainly, the physiological aspects of a mam-

mal that could thrive on air-dried foods without drinking would be very interesting, and would merit the collection of more extensive data than are now available.

CONCLUSIONS AND SUMMARY

Contrary to what has been alleged for some varieties, the mice used in the present experiments are not able to thrive on air-dried foods without drinking. From considerations of the water requirements for the excretion of metabolic end-products it appears doubtful whether any mice could thrive on a normal diet of air-dried materials containing not more than 10 per cent of moisture. When deprived of water the animals develop complete anorexia and when given graded amounts of water after deprivation of the same they consume graded amounts of food. With ordinary stock rations, air-dried, mice consume an average of 1.3 cc. of water for each gram of food. The same relationship between food and water is oberved under conditions of reduced metabolism, as after fasting, or under conditions of greatly increased food consumption, as during lactation. When an attempt is made to diminish the water requirements by reducing the dietary protein and mineral salts to the physiological minimum, practically the same relation of water drunk to ingested food is found, or actually 1.1 cc. of water per gram of food. Inanition fever does not develop in mice deprived of water. Instead, the temperature drops as it has been observed to do by others in the dog, guinea pig and rabbit. The normal body temperature of adult male mice has been found to vary from 36.0° to 40.0°, and to average 37.8°C.

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